Formation of spores (akinetes) in blue-green algae is controlled by many factors. The transformation of vegetative cells into spores in blue-green algae is a morphogenetic process comparable to phenomenon present in all multicellular organisms.

*Modularia spumigena* (Fig. 1a), isolated from the rice fields of Assam, India, produces spores within a short period. In this organism, whole filament (Fig. 1b) becomes completely sporogenous and almost all vegetative cells are converted into spores (Fig. 1c). This formed the main reason for the selection of this organism for the investigations on sporulation.

The sporulation studies were conducted in liquid medium. In all the experiments, only actively growing vegetative filaments (2 to 3 d old) were used as inoculum.
Process of sporulation

In *Nodularia spumigena*, spore development was found to occur in exponentially growing cultures. In the complete HGZ medium, the initiation of sporulation was found to take place on 6th/7th day after inoculation of vegetative filaments in the fresh medium and almost all vegetative cells turned into spores within 30 d. During the process of sporulation, the vegetative cells became enlarged accompanied by the appearance of cytoplasmic granules in them. Following this stage, a thick spore envelope which is brown in colour was formed around the cells, thereby simultaneously resulting in the closure of protoplasmic connections between the developing spores and adjacent vegetative cells.

Normal pattern of spore formation

Under normal conditions, spore differentiation in *Nodularia spumigena* followed a particular pattern. The spore formation always commenced first in the vegetative cells which were located away from heterocysts (i.e., near the mid-point of filament between heterocysts) (Fig. 1b). Later, the spore formation gradually progressed towards heterocysts. In other words, spores under normal conditions always developed centrifugally thereby establishing a gradient of maturity starting away from heterocysts to near
to heterocysts.

A. EFFECT OF VISIBLE LIGHT ON SPORULATION

Light seems to have considerable effect on spore formation (Wolk, 1965; Nichols et al., 1980; Pandey and Talpassayi, 1980; Fernandes and Thomas, 1982). In Anabaena cylindrica, decreasing light intensity resulted in spore formation at lower cell density and vice versa (Nichols et al., 1980). Sutherland et al. (1979) found that addition of exogenous sucrose prolonged the exponential growth phase and concurrently delayed spore differentiation in Nostoc 7524. Thus limitation of energy supply whether in the form of light or sucrose suggested to be a major factor in the induction of spore differentiation.

In the present investigations, influence of different qualities of light on sporulation in Nodularia spumigena was studied both in the presence and absence of phosphate in the medium.

The method of study is presented in the section dealing with material and methods of investigations (Methods II A:b and III).

Results (Ref. Figs. 2 and 3)

In white light, the average number of cells per filament showed a decline starting from 5th day onwards
both in the medium containing phosphate and lacking phosphate, though the number of cells per filament tended to be slightly higher in the medium lacking phosphate (Figs. 2b and 3b). A similar decline in the average number of cells per filament was also observed in red, green and blue lights starting from 5th day after inoculation. However, in the medium containing phosphate, the filaments were shorter when exposed to blue light compared to that in white, red and green lights.

Spores were not formed in dark. In all types of illuminations, both in the presence or absence of phosphate in the medium initiation of spore formation was accompanied by changes in vegetative cell, and they are reported sequentially. The process of spore formation began with enlargement of vegetative cells accompanied by the appearance of cytoplasmic granules. As the spores matured, a thick envelope is formed around them.

Influence of different qualities of light on sporulation in the medium containing phosphate: Compared to sporulation in white, red and green lights, in the initial stages upto 15 d, a slight stimulation in sporulation was observed in blue illumination (Fig. 2). At the end of 30 d, about 90% of the vegetative cells turned into spores in white and red illuminations while the spore formation was about
Influence of different qualities of light on sporulation in the medium lacking phosphate: In all light conditions, sporulation was faster in the medium lacking phosphate than in the medium containing phosphate. Under white illumination, compared to other lights the sporulation was most rapid and by 15th day, about 90% of the vegetative cells turned into spores in the medium lacking phosphate (Fig. 3). In comparison to sporulation in white light, upto 15 d, the sporulation was slower in red, green and blue lights in that order. However, the maximum sporulation about 90 - 93%, was achieved by 20th day in red light and by 25th day in green and blue illuminations. Irrespective of the quality of illumination, about 90 - 93% of sporulation occurred in all illuminations when the organism was grown in the medium devoid of phosphate (Fig. 3a).

Discussion

In the medium containing phosphate, red light promoted rapid sporulation compared to white light (Fig. 2). On the contrary, in the medium devoid of phosphate, white illumination induced rapid sporulation than red light (Fig. 3). Further, blue light was more stimulatory for sporulation than green light in the medium with phosphate.
while the effect of both of these illuminations on sporulation was almost similar in the medium devoid of phosphate. From these results it may be concluded that the effect of quality of illumination on sporulation is influenced by the presence or absence of phosphate in the medium.

Pandey and Talpasayi (1981) studying the influence of quality of light on sporulation in *Nodularia spumigena* (a strain isolated from the rice fields near Banaras, India) in the medium containing phosphate found that green light did not promote sporulation at all while red light, compared to white illumination, was highly stimulatory for sporulation. Furthermore, they also reported that spore formation was inhibited by about 23% in blue light in comparison to that in white light.

A comparison of *Nodularia spumigena* isolated from Assam rice fields (the strain of the present study) with that of the one isolated from rice fields of Banaras indicates that these two strains differ in their responses to the quality of light even in the presence of the medium containing phosphate. It may be worthwhile to mention here that the content of potassium phosphate (dibasic) in the medium used by Pandey and Talpasayi (1981) was 0.035 mg ml⁻¹ whereas it was 0.039 mg ml⁻¹ in the medium used in the present investigations.
B. EFFECT OF TRACE ELEMENTS ON SPORULATION

Trace elements play an important role in the growth of blue-green algae (see Fogg et al., 1973; Carr and Whitton, 1973 and 1983). However, a survey of the literature indicates that not much attention has been paid to investigate the influence of trace elements on sporulation in blue-green algae though much is known in case of bacteria (see Vinter, 1969; Lewis, 1969). Therefore, a study was undertaken to investigate the effects of different micronutrients on sporulation in *Nodularia spumigena*.

Details of the method of study is presented in the section dealing with material and methods of investigations (Methods IIB:i - vii and III).

Results (Ref. Fig. 4)

Removal of trace elements either totally or individually from the medium affected the growth of *Nodularia spumigena* and the filaments appeared unhealthy. In spite of the retarded growth of the alga in the absence of trace elements, appreciable differences in the filament length were not observed in the medium lacking any of the trace elements as compared to that in control (Fig. 4b).

A lag of 15 d, compared to 5 - 10 d in control, was observed in sporulation in the medium lacking micro-
nutrients like molybdenum, copper or cobalt (Fig. 4). Compared to control, up to 20 d, a slight stimulation in sporulation was observed in the medium lacking boron.

It was observed that on 30th day, compared to control, sporulation was lowest in the medium lacking copper followed by that in the medium devoid of zinc, boron, manganese or cobalt (Fig. 4a).

Discussion

Various micronutrients have been reported to influence the growth of blue-green algae (Holm-Hansan, 1968), but it seems that no concerted attempts were made to study their role in sporulation in these organisms.

A general reduction of spore formation in *Nodularia spumigena* was observed in the medium lacking trace elements. A conspicuous percent inhibition of sporulation in the medium lacking copper (53%), zinc (43%), boron (36%), manganese (20%) or cobalt (19%) indicate that these trace elements are important for spore formation. Different degrees of inhibition of spore differentiation upon removal of copper, zinc, boron, manganese or cobalt from the medium also suggest their relative importance in the spore formation processes. It was found that zinc and copper are very important microelements for the promotion of sporulation
in a bacterium, *Bacillus cereus* (see Vinter, 1969).

In *Modularia spumigena*, deficiency of molybdenum in the medium did not inhibit sporulation (inhibition was found to be only about 3%). This finding is in conformity with the result obtained with *Anabaena cylindrica* in which deficiency of molybdenum also did not influence spore formation (Sinclair and Whitton, 1977).

**C. EFFECT OF NITRATE AND PHOSPHATE ON SPORULATION**

A deficiency of combined nitrogen in the medium has been reported to induce spore formation in *Cylindrosperum* sp (Glade, 1914), *Anabaena doliolum* (Singh and Srivastava, 1968; Tyagi, 1974) and *Anabaena circinalis* (Tyagi, 1978). On the contrary, induction of spore formation in *Anabaena* sp (Canabaeus, 1929), *Anabaena fertilissima* and *Anabaenopsis arnoldii* (Reddy, 1976) could take place only when sufficient concentration of nitrate was maintained in the medium.

Phosphate concentration in the medium was found to be an important factor in regulating spore formation in blue-green algae. A deficiency of phosphate has been reported to induce spore formation in *Cylindrospernum* sp (Glade, 1914; Reddy, 1976), *Anabaena cylindrica* (Wolk, 1965), *Aphanizomenon flos-aquae* (Gentile and Maloney, 1969), *Anabaena torulosa* (Fernandes and Thomas, 1982),
Anabaena variabilis and Nostoc linckia (Reddy, 1983d). Kaushik et al. (1971) found that the optimum concentration of phosphate in the medium aids in quicker development of spores in Anabaena doliolium and Fischerella muscicola. On the other hand, phosphate starvation did not stimulate spore production in Nostoc 7524 (Sutherland et al., 1979), Anabaena fertilissima and Anabaenopsis arnoldii (Reddy, 1983d).

It is evident from the information cited above that the presence or absence of combined nitrogen or phosphorus in the medium plays an important role in the regulation of spore formation in blue-green algae, and their influence differs from one species to another. In the present investigation, experiments were conducted to assess the influence of nitrate, ammonia and phosphate on sporulation in Nodularia spumigena.

Details of the experimental procedure are provided in the section dealing with material and methods of investigations (Methods IIB:a viii - x, IIB:c and III).

Results (Ref. Figs. 5 and 6)

On a given day, average number of cells per filament was found to be more or less similar in the complete medium (control) as well as in the medium lacking nitrate
or phosphate or where sodium nitrate was replaced by ammonium nitrate (Figs. 5b and 6b). However, in comparison to control, upto 10 d, the average length of filaments was found to be more in the medium lacking both phosphate and nitrate, though an extensive fragmentation of filaments took place after 10 d.

Influence of sodium nitrate and ammonium nitrate: In the initial period upto 15 d, sporulation was lower in the medium containing ammonium nitrate compared to the spore formation in the medium with or without sodium nitrate (Fig. 5). At the end of 30 d, it was found that, compared to control (i.e., the medium containing sodium nitrate), the percentage of sporulation was lower in the medium lacking sodium nitrate or where sodium nitrate was replaced by ammonium nitrate (Fig. 5a). Further, it was also found that the complete removal of sodium nitrate from the medium was more inhibitory to sporulation than the medium where sodium nitrate was replaced by ammonium nitrate.

Influence of phosphate: Removal of phosphate from the medium increased the rapidity of sporulation compared to that in the medium containing phosphate (control) (Fig. 6). It was observed that in the medium devoid of phosphate, about 90% of the vegetative cells turned into spores by 15 d whereas in the complete medium a similar percentage
of sporulation was achieved by the end of 30 d.

Combined influence of nitrate and phosphate: In complete HGZ medium (i.e., the medium containing both nitrate and phosphate), about 90% of sporulation occurred by the end of 30 d (Fig. 6). However, when the nitrate was eliminated from the medium, by 30 d only about 72% of vegetative cells turned into spores. On the contrary, in the medium lacking phosphate spore formation was hastened and about 90% sporulation occurred by the end of 15 d. In case of the medium in which both nitrate and phosphate were eliminated, 90% of sporulation occurred only by the end of 25 d.

Discussion

In *Nodularia spumigena*, removal of nitrate (i.e., sodium nitrate) from the medium lowered the percentage of sporulation. This indicates that under nitrogen-fixing conditions (i.e., in the absence of combined nitrogen) sporulation is slower compared to that in non nitrogen-fixing conditions. It is possible that the diversion of energy (ATP) for nitrogen fixation processes might have led to slowing down the process of sporulation. In fact, it was found that in bacteria, spore differentiation is an ATP requiring process (see Peberdy, 1980).
A decrease in sporulation percentage was observed in the medium containing ammonium nitrate compared to that in the medium containing sodium nitrate. It should be mentioned here that in the presence of sodium nitrate in the medium, heterocyst formation in \textit{Nodularia spumigena} was not inhibited while the heterocyst differentiation was inhibited to a large extent in the presence of ammonium nitrate in the medium. Since heterocysts were implicated in stimulating spore formation (see Wolk, 1983), the inhibition of heterocyst differentiation in the presence of ammonium nitrate might have resulted in lowering the percentage sporulation in \textit{Nodularia spumigena}.

A deficiency of phosphate in the medium enhanced the rapidity of sporulation compared to control. Phosphate deficient conditions reported to induce spore formation in \textit{Cylindrospermum} sp (Glade, 1914; Reddy, 1976), \textit{Anabaena cylindrica} (Wolk, 1965), \textit{Aphanizomenon flos-aquae} (Gentile and Meloney, 1969), \textit{Anabaena torulosa} (Fernandes and Thomas, 1982), \textit{Anabaena variabilis} and \textit{Nostoc linckia} (Reddy, 1983d). Kaushik et al. (1971) found that the optimum concentration of phosphate in the medium brings about vigorous sporulation in \textit{Anabaena doliolum} and \textit{Fischellerella muscicola}. They also reported that the concentration of phosphate level beyond a particular level inhibits spore differentiation. Kaushik et al. (1971) suggested that the tendency
to sporulate increases with the ageing of cell and once this tendency is fully developed, phosphate starvation simply hastens the onset of sporulation.

D. EFFECT OF pH ON SPORULATION

Blue-green algal distribution in nature is directly or indirectly influenced by pH of the external medium (Fogg et al., 1973). Most of the members of blue-green algae grow in the pH range 7 to 9 (Gerloff et al., 1950; Kratz and Myers, 1955; Granhall, 1970).

Influence of pH on spore differentiation was reported in case of *Nostoc linckia, Anabaena variabilis, Anabaena fertilissima* and *Anabaenopsis arnoldii* grown in complete medium, (Reddy, 1976 and 1983d). It is not known whether the effect of pH on sporulation varies depending on the nutrient availability. Therefore, studies were conducted to investigate the influence of pH on *Modularia spumigena* grown in complete medium as well as in the medium devoid of nitrate or devoid of phosphate.

Studies conducted at pH 8.5 in a particular combination of the medium served as control for that combination of the medium. The details regarding the experimental procedure are presented in the section dealing with material and methods (Methods IIB:b and III).
Results (Ref. Fig. 7)

*Rodularia spumigena* died at pH 12 in complete medium whereas it survived at the same pH in the medium lacking nitrate or phosphate. The number of cells per filament started decreasing after 5 d in all combinations of the medium as well as at all pH conditions (Figs. 7A:b, 7B:d and 7C:f). In complete medium, on a given day, average filament length at pH 5, 6, 10 and 11 was found to be more than that in other pH conditions (Fig. 7A:b). In the medium lacking nitrate, it was found that the average number of cells per filament decreased as the pH increased from 5 to 12 (Fig. 7B:d). In the medium devoid of phosphate, no appreciable change in the average number of cells per filament was observed at all pH conditions tested (Fig. 7C:f).

In complete medium, compared to the rate of sporulation at pH 8.5 (control), the sporulation was more rapid at pH 9 whereas it was slower in pH range from 5 to 8 and 10 to 12 (Fig. 7A). In the medium lacking nitrate, the sporulation was rapid up to 15 d at pH 7, 8, 10 and 11 compared to the sporulation at pH 8.5 (control) (Fig. 7B). On the other hand, in the same medium, in comparison to sporulation at pH 8.5, the sporulation was slower in highly acidic or alkaline conditions (i.e., at pH 5, 6, and 12). In the medium lacking phosphate, the rate of sporulation
was found to be similar at pH 8.5 (control) and 9 whereas it was slower at other pH conditions (Fig. 7C).

In complete medium, by the end of 30 d, a high percentage of sporulation (i.e., more than 85%) was observed in pH range 6 to 9, whereas in the medium lacking nitrate it was between pH 6 to 7 and 10 to 11. In the medium devoid of phosphate a similar percentage of sporulation was achieved in pH range 6 to 12 (Figs. 7A:a; 7B:c and 7C:e). In highly acidic condition (i.e., at pH 5), 66% sporulation was observed in the medium lacking phosphate while it was about 49% in complete medium and 16% in the medium lacking nitrate (Figs. 7A:a, 7B:c and 7C:e). In very alkaline condition, (i.e., at pH 12) sporulation was not observed in complete medium since the organism died (Fig. 7A:a). On the other hand, at the same pH condition the filaments sporulated in the medium lacking nitrate and phosphate and it was found to be more in the medium devoid of phosphate (about 92%) than in the medium without nitrate (about 52%) (Figs. 7B:c and 7C:e).

Discussion

From the results it is evident that at pH 8.5 the percentage of sporulation was lower in the medium lacking nitrate compared to that in complete medium as well as in the medium lacking phosphate. However, it was
observed that in the medium lacking nitrate, pH 6 to 7 and 10 to 11 resulted in increased percentages of sporulation compared to that at pH 8.5 (control) (Fig. 7E). But such a stimulation of sporulation when compared to respective controls, was not observed in the complete medium as well as in the medium lacking phosphate (Figs. 7D and 7F). These findings suggest that the pH optima for sporulation varies depending on the presence or absence of nutrients such as nitrate and phosphate in the medium.

It was found that sporulation occurred, though to a different degrees, in the pH range 5 to 11 in complete medium and 5 to 12 in the medium lacking nitrate and phosphate. These findings indicate that sporulation can take place in almost all pH conditions tested.

Increase of pH higher than required for optimum growth resulted in early spore formation in Nostoc linckia, Anabaena variabilis and Anabaena fertilissima (Reddy, 1983d). However, when Modularia spumigena was grown in complete medium, the maximum percentage of sporulation occurred at pH 8 to 9 which is the pH range for optimum growth in this alga.

E. EFFECT OF AMINO ACIDS ON SPORULATION

Blue-green algae being photoautotrophic organisms
can produce all amino acids which are needed for their
growth (Carr, 1973). Inspite of this, many blue-green
algae were found to photoassimilate the amino acids when
they are supplied in the external medium (see Smith, 1973).
Studies were conducted to investigate the influence of
exogenously supplied amino acids on growth or amino acid
biosynthesis in Anacystis nidulans, Coccochloris penicystis,
Gloeocapsa alpicola, Anabaena cylindrica, Anabaena variabilis
and Nostoc linckia (Maclean et al., 1965; Brownell and
Nicholas, 1967; Smith et al., 1967; Hood and Carr, 1971;
Ladha and Kumar, 1978). In contrast to this, studies concern-
ed with influence of exogenously supplied amino acids
on sporulation in blue-green algae are very limited and
they are confined to investigations in Cylindrospermum
licheniforme (Hirosawa and Wolk, 1979) and Anabaena cylindrica
(Nichols et al., 1980).

Since heterocysts are implicated in regulating
spore formation (see Wolk, 1983), it has been suggested
that products of fixed nitrogen may play an important
role in spore differentiation. Indeed differentiation
of vegetative cell into a spore is characterized by
the proliferation of cynophycin granules (Miller and Lang,
1968; Wildman et al., 1975), a nitrogen reserve material
consisting of an aspartic acid linear polymer with arginine
side chains (Simon, 1971). In the present study, the
Experiments were conducted to examine the effects of exogenously supplied amino acids on spore formation in *Nodularia spumigena* grown in both presence or absence of nitrate in the medium.

The amino acids used in these investigations were L-arginine, L-aspartic acid, L-isoleucine, L-leucine, L-phenylalanine and L-tryptophan. Methods of study are given in the section dealing with material and methods of investigations (Methods IIB:d and III).

**Results** (Ref. Figs. 8 to 13)

Inclusion of L-aspartic acid, L-isoleucine, L-leucine, L-phenylalanine or L-tryptophan at 10 μM level in the medium with/without nitrate resulted in lysis of vegetative cells. On the other hand, vegetative filaments grew well in the presence of similar concentration of L-arginine.

On any given day during the course of experiments, length of the filaments was generally found to be more in the medium lacking nitrate than in the medium containing nitrate. Addition of L-aspartic acid, L-isoleucine, L-leucine, L-phenylalanine or L-tryptophan did not bring about the changes in the filament lengths while L-arginine resulted in a slight increase in filament length (Figs.
Generally the filament length, irrespective of the presence or absence of amino acids, was found to decrease after 5 to 10 d in the medium with or without nitrate.

In the initial period up to 15 d, generally the sporulation percentage was found to be slightly more when the amino acids (up to a concentration of 1 \( \mu \text{M} \)) were exogenously supplied both in the medium containing nitrate or lacking nitrate (Figs. 8A, 9A, 10A, 11A, 12A and 13A). However, at higher concentrations (10 \( \mu \text{M} \)) all amino acids excepting L-arginine, drastically inhibited sporulation both in presence or absence of nitrate in the medium.

In the medium containing nitrate addition of amino acids up to a concentration of 1 \( \mu \text{M} \), did not appreciably affect sporulation (Figs. 8B, 9B, 10B, 12B, 11B, 12B, and 13B). However, an increase in the concentration of L-aspartic acid, L-isoleucine, L-leucine, L-phenylalanine or L-tryptophan to 10 \( \mu \text{M} \) level resulted in lysis of the vegetative cells and hence, sporulation was not observed. On the other hand, the presence of L-arginine at 10 \( \mu \text{M} \) level in the medium supported the growth of the organism and sporulation was found to be almost normal (only about 8% decrease was observed) as compared to control.
In the medium lacking nitrate, exogenous supply of L-leucine decreased the percentage of sporulation as the concentration of the amino acid was increased in the medium (Fig. 11B). In the same medium, addition of L-aspartic acid (0.1 µM) and L-isoleucine (1 µM) slightly increased the percentage of sporulation (Figs. 9B and 10B). On the other hand, inclusion of L-arginine (0.1 to 10 µM) and L-tryptophan (0.1 to 1 µM) stimulated the percentage of sporulation appreciably (Figs. 8B and 13B). Addition of L-aspartic acid, L-isoleucine, L-leucine, L-phenylalanine or L-tryptophan at a level of 10 µM in the medium lacking nitrate led to lysis of the vegetative cells and hence sporulation was not observed. But inclusion of 10 µM L-arginine in the medium stimulated sporulation by 40%.

Discussion

Hirosawa and Wolk (1979) working with *Cylindrospermum licheniforme* (grown in the absence of combined nitrogen) reported that arginine, aspartic acid, phenylalanine and leucine increased sporulation up to a concentration of 16 mM while tryptophan and isoleucine stimulated sporulation up to 1 and 5 mM, respectively. Further, they reported that the most effective amino acid which brought about increased sporulation was tryptophan (1 mM) and it was
followed by phenylalanine (16 mM), aspartic acid (16 mM), isoleucine (5 mM), arginine (16 mM) and leucine (16 mM). In case of Nodularia spumigena (grown in the absence of combined nitrogen in the medium), the most effective amino acid which stimulated sporulation was L-arginine (0.1 to 10 µM) and it was followed by L-tryptophan (1 µM), L-aspartic acid (0.1 µM) and L-isoleucine (1 µM). On the other hand, in Nodularia spumigena, unlike in Cylindrospermum licheniforme, L-phenylalanine (1 µM) did not stimulate sporulation while L-leucine (0.1 µM) inhibited spore formation. Excepting L-arginine all amino acids when added at a concentration of 10 µM resulted in the death of the alga. A comparison of spore formation in Nodularia spumigena and Cylindrospermum licheniforme in presence of amino acids indicates that the response of these organisms towards amino acids differ to a large extent and Nodularia spumigena seems to be more sensitive to high concentrations of amino acids than Cylindrospermum licheniforme.

Nichols et al. (1980) reported that the external supply of arginine did not influence spore formation in Anabaena cylindrica in the medium lacking combined nitrogen.

The observation that low concentrations of L-tryptophan stimulate sporulation gains interest from the fact that among the amino acid analogues tested by Mitchison
and Wilcox (1973), only 7-azatryptophan altered the pattern of spacing of heterocysts in *Anabaena* sp.

From the findings of the present study, it is evident that the influence of externally supplied amino acids differs, at least when they are added at lower concentrations, depending upon the presence or absence of nitrate in the medium. The reason for such a differential effect is not clear.

**F. EFFECT OF GROWTH REGULATORS ON SPORULATION**

Growth regulators such as auxins, cytokinins and gibberellins regulate different morphogenetic and biochemical processes in higher plants (see Leopold and Kriedemann, 1980). Though there is no conclusive proof of production of growth hormones by blue-green algae, the growth hormones secreted by higher plants may influence the growth and development processes of blue-green algae, since in nature, blue-green algae are generally found to grow in association or in the vicinity of higher plants.

The studies concerned with the influence of growth regulators on growth and development of blue-green algae are meagre (see Holm-Hansen, 1968). A limited information pertaining to the effect of growth hormones on hormogonia in *Nostoc* (Bunt, 1961), growth in *Trichodesmium*
erythraeum (Ramamurthy, 1970) and heterocyst differentiation in Anabaena ambiguа (Bahal et al., 1973) is available.

Growth regulators such as auxins, cytokinins, gibberellins regulate seed setting in higher plants (see Leopold and Kriedemann, 1980). It may be of interest to study as to how growth regulators influence sporulation in blue-green algae. In Cylindrospermum majus, gibberellin was found to hasten sporulation (Singh et al., 1965) while in Anabaena dolio{l}um, 2,4-dichlorophenoxy acetic acid delayed spore formation (Srivastava and Tiwari, 1985). In the present investigation, experiments were conducted to study the effects of growth regulators like 2,4-dichlorophenoxy acetic acid (2,4-D), Indole-3-acetic acid (IAA), 6-furfurylaminopurine (Kinetin) and gibberellic acid (GA₃) on sporulation in Nodularia spumigena.

Experiments were conducted in the medium supplemented with one growth regulator at a time. Two sets of experiments were set up to assess the effect of each growth regulator on sporulation. In one set of the experiments, the application of the growth regulator was done at vegetative growth phase (i.e., on 0 d) of the organism while in another set the growth regulator was added to the cultures when the organism reached spore initiation stage (i.e., on 7th day). The methods of investigation are presented in
the section dealing with material and methods of investigations (Methods II B: e i – iv and III).

Results (Ref. Figs. 14 to 21)

In all growth regulator treatments as well as in controls the filament length started decreasing after 5 d (Figs. 14A:b, 15A:b, 16A:b, 17A:b, 18A:b, 19A:b, 20A:b and 21A:b). Generally, in 2,4-D treatments the filament length decreased as the concentration of chemical was increased (Figs. 14A:b and 15A:b). On the contrary, such an effect on filament length was not found with IAA, kinetin and GA₃ (Figs. 16A:b, 17A:b, 18A:b, 19A:b, 20A:b and 21A:b).

Compared to control, in the initial period upto 15 d, generally a slight stimulation in sporulation was observed in the presence of 2,4-D in the medium (Figs. 14A and 15A). On the other hand, in the medium where IAA, kinetin or GA₃ were added there was a general decrease in the rate of sporulation (Figs. 16A, 17A, 18A, 19A, 20A and 21A).

In comparison to controls, on 30th day, 2,4-D upto a concentration of 0.1 μM increased the percentage of sporulation while the concentrations beyond 0.1 μM inhibited it, irrespective of the day of its application (Figs. 14A:a and 15A:a). In case of IAA, its application
at vegetative phase decreased or reduced percentage of sporulation, while its addition at spore initiation stage slightly stimulated sporulation only at lower concentrations (i.e., 0.025 to 0.05 μM) (Figs. 16A:a and 17A:a). Inclusion of kinetin in the medium reduced the percentage of sporulation when applied at both vegetative and sporulation stages (Figs. 18A:a and 19A:a). In case of GA₃, its application up to a concentration of 0.025 μM, whether applied at vegetative stage or at spore initiation stage did not affect sporulation while the concentrations beyond 0.025 μM inhibited the sporulation, though the inhibition was more pronounced when applied at spore initiation stage (Figs. 20A:a and 21A:a).

A comparison of the Figs. 14B, 15B, 16B, 17B, 18B, 19B, 20B and 21B reveals that IAA (0.5 μM) resulted in a maximum percent inhibition (about 60-70%) of sporulation when added both at vegetative and spore initiation stages while GA₃ (0.5 μM) produced such an inhibition when it was supplied only at spore initiation stage of the alga. On the other hand, addition of 0.5 μM of 2,4-D or kinetin resulted in about 20% decrease in sporulation when added at both the stages, while GA₃ showed a similar effect on sporulation in the cultures when it was supplied at vegetative stage only.
Discussion

2,4-D, which is a herbicide and falls under the family of auxins, was found to be a growth stimulator to higher plants when it was supplied in lower concentrations (see Leopold and Kriedemann, 1980). Srivastava and Tiwari (1985) found that addition of 2,4-D in the medium lowered the percentage of sporulation in *Anabaena doliolum*. In the present investigation, it was found that 2,4-D up to a concentration of 0.1 μM stimulated sporulation while higher concentrations were inhibitory. The inhibition of sporulation at higher concentrations of 2,4-D could be due to suppression of protein synthesis (Otter, 1967), a process essential for differentiation in blue-green algae (Talpasayi and Kale, 1967; Kale et al., 1973). In fact, protein content of spores was found to be 3 to 4 folds higher than in vegetative cell in *Anabaena cylindrica* (Simon, 1977a).

In *Nodularia spumigena*, addition of IAA to the medium generally lowered the rate of sporulation. Also in *Cylindrosporum majus*, it was found that sporulation was delayed by the presence of IAA in the medium (Singh et al., 1965). It was reported that in *Cylindrosporum majus*, stimulation of vegetative growth by IAA may be a reason for the reduction of sporulation since spores
are usually formed under adverse conditions (Singh et al., 1965). The increase in filament length in presence of IAA in *Nodularia spumigena* could be due to increased cell division.

In *Nodularia spumigena*, Kinetin and GA₃ slightly inhibited the rate of sporulation. On the contrary, in *Cylindrospermum majus*, it was reported that gibberellin hastened the rate of sporulation (Singh et al., 1965). It is possible that blue-green algae contain endogenous substances like gibberellins (Gupta et al., 1967; N.N. Prasad, personal communication) and an increase in their endogenous level due to the uptake of exogenously supplied gibberellins probably leads to either stimulation or inhibition of spore differentiation depending on the species.

An inhibition of heterocyst differentiation was observed in *Anabaena ambiguа* when grown in the presence of gibberellin (Bahal et al., 1973).

### G. EFFECT OF UNCOUPLER, AND ENERGY AND ELECTRON TRANSPORT INHIBITORS ON SPORULATION

2,4-Dinitrophenol (DNP; an uncoupler), N-N'-dicholorohexylcarbodiimide (DCCD; an energy transfer inhibitor), 3-(3,4-dicholorophenyl)-1,1-dimethylurea (DCMU: and electron transport inhibitor) have been extensively used to understand
different biological process in various organisms. In blue-green algae, effects of DNP on respiratory oxygen uptake in *Synechococcus* sp and *Phormidium luridum* (Biggins, 1969), DCCD on nitrate uptake in *Nostoc muscorum* (Rai et al., 1981) and DCMU on photosynthesis in *Anabaena flos-aquae* (Stewart and Pearson, 1970) were studied. It seems that no investigations have been conducted with these chemicals to study their effects on sporulation in blue-green algae. Hence, studies were undertaken to assess the influence of DNP (an uncoupler of oxidative phosphorylation), DCCD (an energy transfer inhibitor in photophosphorylation) and DCMU (an electron transport inhibitor in photosystem II) on sporulation in *Modularia spumigena*. One of the aims of these investigations was to ascertain the source of ATP generation which contributes to the energy demands of cells when they are differentiating into spores.

Experiments were conducted in the medium supplemented with different concentrations of DNP, DCCD or DCMU. Two sets of experiments were set up to study the effects of each chemical on sporulation. In one set, DNP/DCCD/DCMU was added on 0 d (i.e., at the vegetative growth phase) while in another set they were added on 7th day (i.e., at the spore initiation phase). The method of study is provided in the section dealing with material and methods.
of investigations (Methods II B:e v – vii and III).

Results (Ref. Figs. 22 to 27)

DNP: Addition of DNP did not affect filament length (Figs. 22A:b and 23A:b). Generally, the filament length started decreasing, in presence or absence of DNP, after 5 d of growth.

Compared to control, sporulation was slower in the filaments to which DNP was supplied at vegetative growth phase while such a consistent reduction in the rate of sporulation was not found when DNP was added at spore initiation phase (Figs. 22A and 23A). Addition of DNP (0.5 mM) did not appreciably affect sporulation at 30 d, regardless of the time of application (Figs. 22A:a and 23A:a). But higher concentrations (1 - 3 mM) of DNP was found to be inhibitory (Figs. 22A:a and 23A:a) and percentage inhibition was in proportion to the increase in concentration of DNP in the medium (Figs. 22B and 23B).

DCCD: Both in the presence or absence of DCCD the filament length decreased after 5th day (Figs. 24A:b and 25A:b). Exogenous application of DCCD did not affect the filament length.

When DCCD was added at vegetative growth phase,
it resulted in a slight enhancement in the rate of sporulation upto 20 d compared to control (Fig. 24A). On the other hand, in the same period, such a stimulation in the rate of sporulation was not observed in the cultures in which DCCD was added at spore initiation phase (Fig. 25A). On 30th day, the percentage of sporulation was found to be more in the cultures in which DCCD was added at vegetative growth phase (24A:a). On the contrary, when DCCD was supplied at the spore initiation phase, it resulted in a slight reduction in the percentage of sporulation (Fig. 25A:a). About 12% increase in sporulation was observed when DCCD was added at vegetative phase (Fig. 24A). On the other hand, about 10% inhibition in sporulation occurred in the cultures in which DCCD was added at spore initiation stage (Fig. 25B).

DCMU: Addition of DCMU in the cultures at vegetative phase resulted in the death of the organism (Fig. 26A:b). On the other hand, when DCMU was added to the cultures at spore initiation stage, the organism survived to some extent, and the length of the filaments was found to be similar both in the presence or absence of the chemical (Fig. 27A:b).

Inclusion of DCMU at vegetative growth phase resulted in the death of organism and consequently sporulation
was not observed (Fig. 26A and B). When DCMU was added to the cultures at the spore initiation phase, there was a reduction in the rate of sporulation (Fig. 27A). By the end of 30 d, fall in the percentage of sporulation, compared to control, was drastic when DCMU was added at a concentration of 1.25 μM (Fig. 27A:a). Further increase in concentration of DCMU in cultures resulted in a gradual reduction in percentage of sporulation. About 70% inhibition of sporulation was observed in the presence of 1.25 to 5 μM DCMU and the percentage inhibition reached to about 90 when the concentration of DCMU was increased to 20 μM (Fig. 27B).

Discussion

Under normal conditions, in the vegetative filaments of *Nodularia spumigena*, between 5 to 6 d after inoculation, ATPase activity decreased in those cells which would differentiate into spores (see page 124). Decrease in ATPase activity suggests low ATP generation in the cells. Lowering of ATP production to a particular threshold level probably triggers differentiation of spores in *Nodularia spumigena*. A role for ATP in initiation of sporulation in a bacterium *Bacillus subtilis* has been put forward (see Peberdy, 1980). In *Bacillus subtilis*, since the level of ATP falls at the end of exponential phase it is suggested that the
depletion of ATP is the signal for spore development. When ATP is maintained at high level as in growing cells, sporulation is blocked.

In the present study, it was found that about 20 to 24% of inhibition of sporulation occurred in presence of 3 mM DNP, regardless of its addition either at vegetative phase or at spore initiation phase (Figs. 22B and 23B). This indicates that the maximum concentration of DNP used in the cultures moderately affected sporulation. Such a moderate inhibition of sporulation in the presence of DNP (3 mM) suggests that the energy (ATP) needed for sporulation processes is probably derived, at least partly, from oxidative phosphorylation; since the presence of 1 to 3 mM DNP inhibits the generation of ATP through oxidative phosphorylation process by specifically uncoupling the ATPase system from phosphorylating electron flow (Newmann and Jagendorf, 1964; see Lehninger, 1976).

Application of DCCD at two different phases (i.e., at vegetative phase and spore initiation phase) showed contrasting effects on sporulation in *Nodularia* *spumigena*. When DCCD was applied at vegetative phase, it resulted in about 12% stimulation in sporulation while its application at spore initiation phase brought about 10% inhibition in spore formation.
In *Nodularia spumigena*, stimulation of sporulation in the initial period of growth in the cultures in which DCCD was applied at vegetative stage (Fig. 24A) may be due to the advancement (i.e., lowering of ATP production earlier than 5 - 6 d, a normal period at which ATPase activity diminishes in those cells which would differentiate into spores) of reduction in ATP generation in initial growth phase as the result of DCCD application; since DCCD inhibits photophosphorylation by blocking free energy transfer from photosynthetic electron flow to phosphorylating ATPase system (McCarty, 1980). About 12% increase in sporulation in these cultures (Fig. 24B) may be due to the cumulative effect of reduction in ATP production which might have resulted from DCCD application as well as lowering of ATPase activity during normal course in the differentiating cells. On the other hand, inhibition of sporulation when DCCD was added at spore initiation phase is probably due to the reduction of ATP production in the cells below a certain threshold level which may be inhibitory to spore differentiation processes.

In *Nodularia spumigena*, it was found that the addition of DCMU at vegetative growth phase resulted in the death of the organism (Fig. 26B). This is evidently due to the inhibition of photosynthesis because DCMU stops
the production of ATP and NADPH$_2$ by inhibiting non-cyclic electron flow in photosynthetic process (see Izawa and Good, 1972). Addition of DCMU to the cultures at the spore initiation phase brought about a drastic reduction (up to 90% inhibition) in sporulation (Fig. 27B). This clearly indicates that the inhibition of photosynthesis by DCMU affects sporulation in _Nodularia spumigena_. From this it may be suggested that fresh synthesis of carbohydrates is necessary for spore differentiation. Fay (1969b) reported that in _Anabaena cylindrica_, during spore formation, photosynthesis continues at a gradually decreasing rate which results in several fold increase in dry weight probably due to the increase in reserve materials. In _Nodularia spumigena_, during the course of present investigations, it was found that the carbohydrate content gradually increased in the developing spores (see page 117) which is probably due to continuous but low rate of photosynthetic activity going on in these cells.

Cell differentiation is an energy (ATP) requiring process (Peberdy, 1980). In _Nodularia spumigena_, the results pertaining to the studies on sporulation in presence of DNP and DCCD suggest that energy required for spore differentiation is not solely met by a single phosphorylating system and both oxydative- and photophosphorylation processes:
contribute to the energy demand of the process. At the same time, the contribution of ATP needed for sporulation process by tricarboxylic acid cycle cannot be ruled out as the energy needed for sporulation in *Bacillus subtilis* is suggested to be derived from tricarboxylic acid cycle (see Perberdy, 1980). Studies are needed to determine the magnitude of ATP contribution by different ATP generating process for sporulation in blue-green algae.

The investigations on sporulation in presence of DCMU suggest that a fresh synthesis of carbohydrates is essential for spore formation.
In *Nodularia spumigena*, vegetative cells differentiate into heterocysts and spores at certain stage of growth. Under normal conditions, heterocyst and spore differentiation followed a particular pattern. Heterocyst formation took place more or less at regular intervals in a filament. Spore formation always commenced first in the vegetative cells which were located away from heterocysts i.e., near the mid-point of the filament between heterocysts, and it gradually progressed towards heterocysts. So far no studies have been conducted to understand as to how the spacial pattern of spores is organized in filaments of blue-green algae. To achieve this end, investigations were conducted to get in insight into biochemical changes taking place in vegetative cells in intact filaments before and during their transformation into spores.

A. CYTOCHEMISTRY OF SPORE DIFFERENTIATION

Variations in the levels of catalase, peroxidase, glutamine synthetase, ATPase, and acid and alkaline phosphatases were studied employing cell-free extracts of sporeulating filaments of *Anabaena torulosa* (Sarma and Kanta, 1982). Though such a study using cell-free extracts provides an information with regard to the biochemical changes taking place during sporulation, it does not give an idea about
where exactly these changes are taking place in the filaments. To achieve this, in the present study, cytochemical methods were employed to identify the sites (cells) of biochemical changes occurring during sporulation in intact filaments; as such an information forms a basis to understand the processes which regulate pattern of spore differentiation.

In the present investigation, the studies were mainly concentrated on the localization of changes taking place in polysaccharide/carbohydrate, ascorbic acid, arginine, tryptophan and polyphosphate contents, and L-aspartate:2-oxoglutarate aminotransferase (EC 2.6.1.1), esterase (EC 3.1.1.1), peroxidase (EC 1.11.1.7), phosphorylase (EC 2.4.1.1), alkaline phosphatase (EC 3.1.3.1), acid phosphatase (EC 3.1.3.2), glucose-6-phosphatase (EC 3.1.3.9), adenosine triphosphatase (EC 3.6.1.3) and TTC reducing activities in vegetative (2-3 d old) as well as sporulating (at both spore initiation stage, i.e., on 5-7 d and spore maturation stage, i.e., beyond 8 d) filaments. Details of the experimental protocols followed in these studies are presented in the section dealing with material and methods of investigations (Methods VIII A-N).

Results (Ref. Figs. 28 to 38)

Polysaccharides/Carbohydrates: Young vegetative filaments, when stained with PAS reagent, showed an uniform pink
colouration in all vegetative cells (Fig. 28 a) thereby indicating similar content of polysaccharides in all cells. At a later stage, before any morphological changes accompanying spore differentiation could be detected in filaments, a gradient in polysaccharide staining was observed in filaments, with the most intense staining in the vegetative cells next to heterocysts and intensity of staining gradually diminishing with increasing distance from heterocysts (Fig. 28 b,c). In developing spores, intensity of staining was found to be more as compared to adjacent vegetative cells (Fig. 28 d-h).

Staining of vegetative filaments (2-3 d old) for total carbohydrates with IKI solution (prepared by dissolving 2 g of potassium iodide and 0.2 g of iodine in 100 ml of double distilled water) exhibited an uniform intensity of stain in all vegetative cells (Fig. 28 i) indicating similar content of carbohydrates in the cells. In comparison to the intensity of staining at young vegetative growth stage, the intensity of staining in the cells when they were under the process of differentiation was less and discontinuous (Fig 28 j). In later stages, as spores matured an intense staining was noticed thereby indicating a high carbohydrate content in them (Fig. 28 k).

Ascorbic acid: When young vegetative filaments (2-3 d old) were stained with silver nitrate reagent, vegetative cells
showed very less deposit of silver precipitation in them, suggesting low content of ascorbate in those cells (Fig. 29 a). The intensity of staining in the cells which are initiated for spore differentiation was always very high (Fig. 29 b-d) compared with non-differentiating vegetative cells (Fig. 29 a). A dark violet coloured deposit of silver was observed in maturing as well as matured spores (Fig. 29 d-f) suggesting the presence of ascorbic acid in them.

Arginine: Staining of 2-3 d old vegetative filaments did not reveal arginine containing granules in them (Fig. 30 a). However, 5-6 d old filaments when stained, showed the accumulation of arginine in the cells which are close to the centre of interheterocyst interval (Fig. 30 b,c). It was found that arginine content of the developing spores increased with the increasing maturity (Fig. 30 d-g).

Tryptophan: Young filaments (2-3 d old) when stained for the presence of tryptophan, they did not show the reaction in vegetative cells (Fig. 31 a). On the other hand, 5-6 d old filaments showed the accumulation of tryptophan in the cells which are located near the mid-point of filament between two heterocysts (Fig. 31 b-d). In a later stage, all vegetative cells in filaments showed the accumulation of tryptophan in them (Fig. 31 e-g).
Tryptophan containing granules could be located in cells at all different stages of spore differentiation as well as in matured spores (Fig. 31 h-m).

Polyphosphate compounds: In initial stage of growth, polyphosphate content of vegetative cells (in 2-3 d old filaments) was found to be generally uniform throughout filament length (Fig. 32 a). In 5 d old filaments, a gradient of polyphosphate material developed, with the most polyphosphate present in the vegetative cells next to heterocysts and the amount of polyphosphate present per cell gradually diminishing with increasing distance from heterocysts (Fig. 32 b,c). It was observed that differentiating cells containing less polyphosphate material in them compared to the adjacent vegetative cells (Fig. 32d). A gradual loss of polyphosphate material took place as spores matured and ultimately fully matured spore lacked polyphosphate granules (Fig. 32 e,f). The lack of staining in such mature spores may not be due to impermeability of spore wall as protoplasts of spores also failed to show polyphosphate material (Fig. 32 g).

When the broken or fragmented filaments were stained for polyphosphates, they showed intense staining in the vegetative cells adjacent to heterocysts as compared to the cells which were located near the cut ends (Fig. 32
This finding strongly evidences that the intense staining of polyphosphates near heterocysts is not due to a consequence of localized penetration of the stain at connections between heterocysts and vegetative cells.

**TTC reducing activity:** When young (2-3 d old) filaments were treated with TTC, the indicator was reduced rapidly (within 30 min) in heterocysts, which showed dense deposition of red formazan crystals (Fig. 33 a). In sporulating filaments, formation of formazan crystals due to the reduction of TTC was more in the vegetative cells which are near heterocysts as compared to those vegetative cells which are located away from heterocysts (Fig. 33 c,d). It was also found that the intensity of TTC reducing activity increased in the cells which are being developed into spores (Fig. 33 b-d). Further, matured spores showed conspicuous deposition of formazan crystals in them, thereby suggesting the development of highly reduced conditions in spores (Fig. 33 b-e).

**L-aspartate:2-oxoglutarate aminotransferase activity:** When vegetative filaments (2-3 d old) were stained for L-aspartate:2-oxoglutarate aminotransferase activity, it was found that the enzyme activity was more or less uniform in all vegetative cells (Fig. 34 a). In 5 d old filaments, even before any morphological changes leading
to spore differentiation could be detected, the enzyme activity was found to be low in the cells which are located near the mid-point of filament (where spores differentiate first) between two heterocysts and the activity progressively increased towards heterocysts (Fig. 34 b). However, during spore formation, gradient of the enzyme activity was found to be reversed and it was more in the cells which are developing into spores and less in the vegetative cells which are situated near to heterocysts (Fig. 34 c,d). In a later stage, as spores matured the enzyme activity in them gradually decreased and ultimately no enzyme activity was observed in completely matured spores (Fig. 34 c,d).

Esterase activity: In 2-3 d old filaments esterase activity, indicated by a dark brown reaction product, was found to be uniform in all vegetative cells (Fig. 35 a). In sporulating filaments, the intensity of enzyme activity decreased compared to that in young vegetative filaments and it was observed that the activity of enzyme was low in differentiating cells (Fig. 35 b-e). Esterase activity was not detected in matured spores (Fig. 35 b-e).

Peroxidase and Phosphorylase activities: At no stage of growth or development peroxidase as well as phosphorylase activities were detected in filaments.

Alkaline phosphatase activity: Vegetative cells did not
exhibit alkaline phosphatase activity though the enzyme activity, indicated by the formation of reddish/brown reaction product, could be detected in mucilagenous sheath of filaments (Fig. 36 a). However, during the course of maturation of spores, alkaline phosphatase activity was detected in cell membrane of developing spores (Fig. 36b).

Acid phosphatase activity: Vegetative filaments, 2-3 d old did not exhibit acid phosphatase activity in cells. In 8 d old filaments, acid phosphatase activity, indicated by the deposition of reddish/brown reaction product, was observed in the cells which are initiated for spore differentiation (Fig. 36 c,d). In later stages of the development, as spores matured the enzyme activity gradually decreased and finally no activity was observed in fully matured spores (Fig. 36 e).

Glucose-6-phosphatase activity: When young vegetative filaments (2-3 d old) were stained for glucose-6-phosphatase activity, it was found that an uniform deposition of brownish black lead sulphide precipitation occurred in all vegetative cells indicating the enzyme activity was similar in the cells (Fig. 37 a). In sporulating filaments, the enzyme activity gradually became intense upto a certain stage of spore differentiation (Fig. 37 b-e). Later, the enzyme activity was found to decrease in maturing spores and matured spores did not show any activity in them (Fig. 37 f).
Adenosine triphosphatase (ATPase) activity: In 2-3 d old filaments, ATPase activity was found to be uniform in all vegetative cells (Fig. 38 a). In 5 d old filaments, it was observed that a gradient of ATPase activity developed, with a high enzyme activity in the vegetative cells next to heterocysts and its activity progressively diminishing with the increasing distance from heterocysts (Fig. 38 b,c). In sporulating filaments, the activity was low in the cells which are being differentiated into spores and matured spores did not show any enzyme activity in them (Fig. 38 d).

Discussion

Electron microscopic studies revealed that spore formation in blue-green algae is accompanied by increasing compactness of protoplast, condensation of photosynthetic thylakoids with decreased interthylakoidal spaces, disappearance of gas vesicles, increase in number of ribosomes, cyanophycin and polyglucon granules, and decrease in number of lipid droplets (Ris and Singh, 1961; Clark and Jensen, 1969; Jensen and Clark, 1969). Further, there is a considerable evidence to show that the chemical constitution of matured spores is different from that of vegetative cells (see Nichols and Adams, 1983; Rai et al., 1985). The results of the present investigations reveal that cells in filaments of *Modularia spumigena* exhibit many
biochemical changes not only during spore development but also before/during initiation of differentiation. It was found that even before morphological changes accompanying spore differentiation could be observed, various biochemical changes take place in the vegetative cells; these include decrease in polysaccharide and polyphosphate contents, and ATPase, L-aspartate:2-oxoglutarate aminotransferase, esterase and TTC reducing activities and increase in arginine and tryptophan contents. However, L-aspartate:2-oxoglutarate aminotransferase and TTC reducing activities are restored in developing spores. Further, the biochemical changes accompanying the morphological changes associated with early stages of spore development are increase in polysaccharide (after initial low) ascorbic acid, arginine and tryptophan contents and alkaline phosphatase, acid phosphatase and glucose-6-phosphatase activities. In matured spores, activities of all enzymes were found to be completely reduced while high TTC reducing capacity was retained.

Presence of a large number of cyanophycin granules in matured spores has been reported by several workers (Miller and Lang, 1968; Lang and Fisher, 1970; Sutherland et al., 1979). Simon (1971) found that the main components of cyanophycin granules are arginine and aspartic acid which contribute to 98% of the dry weight of the granules. It was suggested that cyanophycin granules serve as a
store of combined nitrogen source in spores (Fritsch, 1945; Simon, 1971).

In *Nodularia spumigena*, it was observed that the activity of L-aspartate:2-oxoglutarate aminotransferase decreased in the vegetative cells which are located at a place in filament where spore differentiation commences. In other words, the activity of L-aspartate:2-oxoglutarate aminotransferase decreased in vegetative cells even before any morphological changes associated with spore differentiation took place in them. Since the decrease in the activity of L-aspartate:2-oxoglutarate aminotransferase enzyme leads to a decrease in the production of aspartic acid it seems that the reduction in aspartic acid production to a certain threshold level is essential for promotion of spore initiation processes in vegetative cells. This suggestion gains support from our previous studies where it was found that exogenous supply of high concentrations of aspartic acid in medium inhibited sporulation while low concentrations slightly stimulated it (see page 100).

Histochemical studies showed that L-aspartate:2-oxoglutarate aminotransferase activity was restored in the cells at a certain stage of spore development. Amino acid analysis of matured spores of *Nodularia spumigena* revealed the presence of aspartic acid in them (see Table 5.
and Fig. 40). The accumulation of aspartic acid in matured spores might have resulted from the restoration of the activity of L-aspartate:2-oxoglutarate aminotransferase in the cells during their development into spores following the initiation phase.

In *Nodularia spumigena*, accumulation of arginine and tryptophan in vegetative cells preceding the commencement of morphological changes associated with spore differentiation probably suggests the involvement of these amino acids in metabolic pathways leading to the initiation of spore differentiation. It is worthwhile to mention here that the exogenous supply of arginine (10 μM) or tryptophan (1 μM) in the medium lacking nitrate stimulated sporulation in *Nodularia spumigena* (see page 100). It was found that in *Anabaena cylindrica*, arginine analogues (but not arginine) like canavanine and cyanoalanine, increased spore formation (Nichols et al., 1980) while tryptophan stimulated spore production in *Cylindrospermum licheniforme* (Hirosawa and Wolk, 1979a). Sutherland et al. (1979) reported that in *Nostoc* 7524, pattern of spore formation is altered in presence of 7-azatryptophan, an analogue of tryptophan. Further studies are needed to ascertain the roles of arginine and tryptophan in spore differentiation.
In *Anabaena cylindrica* (Fay 1969b) and *Nostoc spongioforme* (Thiel and Wolk, 1983), a gradual decrease in the rate of photosynthesis was observed during spore formation. In *Modularia spumigena*, it was observed that in 5 d old filaments the polysaccharide content showed a gradient, with most polysaccharide present in the vegetative cells next to heterocysts and their content progressively decreasing with increasing distance from heterocysts. Such a reduction of polysaccharide content in the cells located away from heterocysts indicate lowering of photosynthetic activity in them. The loss of photosynthetic activity seem to precede/accompany the spore initiation process. After a certain period following the cells are committed to differentiate into spores, polysaccharide content was found to increase in these cells; probably because of low but continuous photosynthetic activity in them. Indeed Fay (1969b) reported that during the spore formation photosynthesis in intact sporulating filaments continue at a gradually decreasing rate which results in several fold increase in dry weight.

In *Anabaena fertilissima*, a gradual decrease of polyphosphate material as the spore mature led to the suggestion that loss of polyphosphate from vegetative cells initiates spore formation (Reddy, 1983a). This suggestion gains support from the finding of the present study
with *Nodularia spumigena* which clearly showed a gradual disappearance of polyphosphate from vegetative cells even before any morphological changes associated with spore differentiation could be detected in them. Disappearance of polyphosphate material from the cells situated in the interheterocyst interval where spore differentiation normally commences suggests that the depletion of polyphosphate is associated with the initiation of spore differentiation. It is worthwhile to mention here that during sporulation studies it was found that phosphate starvation hastened spore formation in *Nodularia spumigena* (see page 90).

Sarma and Kanta (1982) reported that in *Anabaena torulosa*, ATPase activity decreased in sporulating filaments. However, in the present investigation it was found that ATPase activity decreased in the cells even before any morphological changes accompanying spore differentiation could be observed. Since ATPase is a reversible enzyme which regulates the formation of ATP, a reduction in its activity probably leads to the depletion of ATP in the cells. In a bacterium, *Bacillus subtilis*, it was found that ATP level was reduced at the end of the exponential growth phase before entering sporulation stage (see Peberdy, 1980). It was also found that when ATP was maintained at high level, as in growing cells, sporulation was blocked. Based on these findings it is suggested that the depletion
of ATP level in the cells of *Bacillus subtilis* is the signal for initiating spore formation mediated through a phosphorylated protein functioning as an aporepressor.

Fay and Kulasooriya (1972) found in *Anabaena variabilis*, a gradient of TTC reducing activity in the vegetative filaments, with highest activity in the vegetative cells present adjacent to heterocysts and the activity progressively decreasing with increasing distance from heterocysts. In *Nodularia spumigena*, a similar gradient of TTC reducing activity developed in vegetative cells between two heterocysts before spore differentiation. But the activity was restored when the cells started developing into spores and subsequently the matured spores had a high TTC reducing activity in them. TTC being a redox dye accepts electrons from electron donors and gets reduced giving rise to red coloured formazan. The intensity of this reaction is proportional to general electron transport activity in cells. Less formazan formation in vegetative cells may be a consequence of reduced electron transport activity in these cells because of lowering of photosynthesis at spore initiation stage while more formazan formation in developing spores may be a consequence of increased electron transport activity due to high respiratory metabolism in these developing cells. Indeed Fay (1969a) reported reduced photosynthetic and increased respiratory activities
in developing spores. In *Nodularia spumigena*, reduced polysaccharide content preceding/associated with spore initiation phase suggests lowering of photosynthetic activity in the vegetative cells even before they started turning into spores. Further, the accumulation of ascorbic acid in developing spores probably forms a basis for increased respiratory metabolism in differentiating spores. However, critical studies are needed before arriving at any conclusion regarding these speculations. In *Cylindrospermum licheniforme*, spore formation is suggested to be controlled by 'some substance' which is reduced by uptake hydrogenase localized in heterocysts (Hirosawa and Wolk, 1979a).

Both phosphorylase and peroxidase activities could not be localized in cells at any stage of growth and development of *Nodularia spumigena*. However, Sarma and Kanta (1982) found that in *Anabaena torulosa* peroxidase showed a higher initial activity at sporulation stage around 8 d and subsequently the activity returned to the level found in vegetative cells within next 4 d.

In *Nodularia spumigena*, a gradual loss of polyphosphate content was observed as spore mature and ultimately fully matured spores lacked polyphosphate material. Development of detectable activities of alkaline and acid phosphatase including glucose-6-phosphatase in developing
spores suggest that these enzymes may be performing a role of removing phosphate from differentiating spores. Specifically, increased glucose-6-phosphatase activity probably indicates an enhancement in the reaction leading to hydrolytic cleavage of glucose-6-phosphate into glucose and inorganic phosphorus (see Lehninger, 1976). In *Anabaena torulosa* also an increase in alkaline and acid phosphatases levels was observed in sporulating filaments (Sarma and Kanta, 1982).

In *Modularia spumigena*, reduction in the activity of general esterases in developing spores suggests the lowering of activities at least of some esterases which bring about hydrolysis of phosphoric and carboxylic esters in these cells.

In *Anabaena cylindrica*, it was found that exogenously supplied ascorbic acid influences development of long strings of spores (Wolk, 1965). During spore formation in *Modularia spumigena*, differentiating cells were found to accumulate high amounts of ascorbic acid compared to vegetative cells. Further, the studies also revealed the presence of ascorbic acid in matured spores. It is difficult to say, at present, what role ascorbic acid plays in the metabolism of spores. It may take an active part in the respiratory metabolism of spores as it was shown that
oxygen uptake continues even in matured spores (Fay, 1969b; Thiel and Wolk, 1983; Rao et al., 1984).

B. PATTERN OF SPORE FORMATION

The position of spores in the filaments of blue-green algae is variable but it is related to the position of heterocysts. The spatial relationship between spores and heterocysts has prompted many workers to implicate heterocysts in spore development (Carter, 1856; Fritsch, 1951; Wolk, 1965 and 1966; Tyagi, 1974). However, the development of spores was also found to occur in complete absence of heterocysts (Eberly, 1966; Hill, 1970; Sutherland, et al., 1979) indicating non involvement of heterocysts in spore formation.

In Nodularia spumigena, frequency of heterocysts is similar both in the presence or absence of sodium nitrate in medium. Further, under both these conditions, spore formation always commenced first at the mid-point of filament between two heterocysts and it gradually progressed towards heterocysts (this type of spore formation henceforth referred to as normal pattern of spore differentiation). However, during sporulation experiments, when the organism was grown in the presence of ammonium nitrate, 2,4-dinitrophenol or N,N'-dicyclohexylcarbodiimide, often it was observed that spore formation commenced adjacent to
heterocysts and it gradually progressed towards the centre of interheterocyst interval (this type of spore formation henceforth referred to as abnormal or altered pattern of spore differentiation; Fig. 39d). Such variations are very useful in understanding the precise role of heterocysts in the regulation of pattern formation of spores. Hence investigations were undertaken by employing chemicals like rifampicin, ammonium nitrate, 2,4-dinitrophenol (DNP), N,N'-dicyclohexylcarbodiimide (DCCD) and N-methyl-N'-nitro-N-nitrosoguanidine (NTG; a chemical mutagen) which alter cellular differentiation in *Nodularia spumigena*. Observations were scored for normal and abnormal patterns of spore differentiation. Cytochemical methods were employed to localize polyphosphate , and L-aspartate:2-oxoglutarate aminotransferase, ATPase and TTC reducing activities in filaments obtained from ammonium nitrate, DCCD and NTG treatments. Details of the experimental procedures are given in the section dealing with material and methods of investigations (Methods VI, VII and VIII E,F,H,N).

**Results** (Ref. Tables 1-3 and Figs. 32-34, 38 and 39)

**Rifampicin:** Filaments of *Nodularia spumigena* died in the presence of 0.6 - 1.2 μM rifampicin. However, supplementing the medium with rifampicin upto a concentration of 0.24 μM resulted in increased frequency of heterocyst
formation from about 5% (control) to 12% while it led
to decrease in percentage of spores from about 91% (control)
to 6% (Table 1). Further, it was found that the decrease
in percentage of sporulation was proportional to the concen-
tration of rifampicin in medium. Pattern of spore differen-
tiation was not affected by the presence of rifampicin
in medium and it was found to be similar to that in control
(Table 2).

Ammonium nitrate: Both normal and abnormal patterns of
spore formations were observed in filaments grown in the
medium containing ammonium nitrate. It was found that
the frequency of abnormal pattern of spore differentiation
increased with the increase in incubation period and it
reached a maximum of about 19% at the end of 30 d (Table 2).
On 30th day, in ammonium grown cultures, about 53% of
filaments exhibited exclusively normal pattern of spore
differentiation while about 13% of filaments showed only
abnormal pattern (Table 3). On the other hand, about 33%
of filaments produced both normal and abnormal types of
spore formations (Table 3 and Fig. 39 b).

Compared to polyphosphate content of vegetative
cells in control (Fig. 32 a), polyphosphate concentration
in vegetative cells of ammonium grown filaments was observed
to be low, though it was uniformly distributed in all
the cells (Fig. 32 j,k). TTC reducing activity was never observed in vegetative cells and heterocysts of ammonia grown filaments while a few spores exhibited TTC reducing activity in them (Fig. 33 f). L-aspartate:2-oxoglutarate aminotransferase (Fig. 34 e) and ATPase (Fig. 38 e) activities were found to be uniform in vegetative cells of ammonia grown filaments. At no stage of growth or development gradient formation (between heterocysts) of either polyphosphates or enzyme activities were observed in these filaments.

DNP: Both normal and abnormal patterns of spore formations were observed in filaments grown in the medium supplemented with DNP. The frequency of abnormal pattern of spore formation was found to be about 6% (Table 2).

DCCD: In the presence of DCCD, filaments exhibited abnormal pattern of spore differentiation, and its frequency was found to increase from about 5% to 11% as the concentration of DCCD was increased from 0.5 μM to 1.0 μM (Table 2).

Cytochemical localization of polyphosphates in filaments grown in the presence of DCCD showed discontinuous accumulation of polyphosphate material in filaments (Fig. 32 l,m). Sometimes vegetative cells present adjacent to heterocysts were found to be lacking polyphosphates (Fig. 32 m) Vegetative cells, heterocysts and spores exhibited TTC
reducing activities (Fig. 33 e). L-aspartate:2-oxoglutarate aminotransferase activity (Fig. 34 f) was found to be uniform in vegetative cells of filaments grown in the presence of DCCD. ATPase activity was very low though it was observed to be uniform in filaments grown in the presence of DCCD (Fig. 38 f) as compared to that in control (Fig. 38 a). Unlike in filaments in controls, at no stage of growth and development gradients of polyphosphates, and enzyme and TTC reducing activities were observed in filaments, though TTC reducing activity was found to increase later in developing spores (Fig. 33 e).

NTG: Filaments in cultures developed from NTG treated material exhibited both normal and abnormal patterns of spore differentiation (Fig. 33 g and 38 h,i). It was observed that the frequency of abnormal pattern of spore formation increased with the increase in incubation period and it reached a maximum of about 19% at the end of 30 d (Table 2). On 30th day, about 47% of filaments exhibited exclusively normal pattern of spore formation while about 23% of filaments showed only abnormal pattern formation (Table 3). On the other hand, about 30% of filaments produced both normal and abnormal types of spore formations (Table 3 and Fig. 38 h).

In NTG treated material, distribution of polyphos-
phates in filaments was found to be ununiform (Fig. 32 n,o). Further, many filaments exhibited less polyphosphate material in vegetative cells adjacent to heterocysts (Fig. 32 n,o). TTC reducing activity was found to be more or less uniform in vegetative cells of young filaments (Fig. 33 g), though the activity rapidly disappeared in old filaments (Fig. 33 h). However, developing spores did show more TTC reducing activity. L-aspartate:2-oxoglutarate aminotransferase activity was found to be similar to that exhibited by the filaments in control (i.e., even before the commencement of spore development could be observed the enzyme activity showed a gradient and it was found to be low in the vegetative cells located near the mid-point of filament between two heterocysts and the activity progressively increased towards heterocysts. However, during spore formation, gradient of the enzyme activity was reversed and it was found to be less in vegetative cells adjacent to heterocysts and more in developing spores; Fig.34 g). ATPase activity in NTG treated materials was found to be discontinuously distributed in filaments (Fig. 38 g-i). On some occasions vegetative cells present adjacent to heterocysts did not exhibit ATPase activity (Fig. 38 g,i).
Discussion

Rifampicin, which is an inhibitor of DNA dependent RNA polymerase in *Escherichia coli* (Lancini *et al.*, 1969), induced differentiation of strings of multiple heterocysts in *Anabaena variabilis* (Wolk and Quine, 1975). However, its site of action at molecular level was not ascertained in case of blue-green algae. In the present study, rifampicin was employed to find out whether alteration of heterocyst frequency has any effect on spore differentiation. In *Rodularia spumigena*, rifampicin induced differentiation of double heterocysts (Fig. 39 e) as well as increase in the number of single heterocysts (Fig. 39 f) which are closely spaced in filaments. On the other hand, although the pattern of spore differentiation was not altered, sporulation was found to be inhibited to a very large extent in the presence of rifampicin. In *Modularia spumigena*, decrease in interheterocyst distance seem to inhibit spore formation. However, possibility of direct effect of rifampicin on sporulation *per se* cannot be ruled out.

In *Modularia spumigena*, both in the presence or absence of sodium nitrate, sporulation in filaments commenced near the centre of interheterocyst interval and gradually progressed towards heterocysts. However, in cultures which were grown in the presence of ammonium nitrate, spore differentiation took place at random positions. Further,
in the initial phase upto 15 d, a simultaneous decrease in spore (see Fig. 5) and heterocyst development was observed. In later stages as more heterocysts developed in filaments, spore differentiation was also enhanced. In other words, when the heterocysts were few in filaments, spore differentiation was low, and as the frequency of heterocysts increased, spore formation was accelerated. These results suggest that in *Nodularia spumigena*, spore formation seems to be regulated/influenced by the presence or absence of heterocysts in filaments.

Incidence of abnormal pattern of spore differentiation in the presence of DNP and DCCD may be because of decreased ATP production (as a result of the action of these chemicals on oxydative- and photophosphorylations) in vegetative cells located adjacent to heterocysts.

Unlike the filaments from controls, filaments from ammonia, DCCD and NTG treatments did not exhibit the formation of gradients of polyphosphates/ATPase activity/TTC reducing activities in them. Since gradient formation of polyphosphates/ATPase activity/TTC reducing activity in filaments was observed to be a prerequisite to maintain regularity in spacing of spore differentiation (see pages 12-13), its absence in filaments from ammonia, DCCD and NTG treatments might have resulted in the formation of spores at
random positions in filaments.

Cytochemical analysis showed uneven distribution of polyphosphate material in filaments from DCCD treatment and both polyphosphate content and ATPase activity in filaments from NTG treatment. Further, it was also observed that sometimes vegetative cells present adjacent to heterocysts lacked polyphosphates and ATPase activity. Commencement of spore differentiation in vegetative cells present adjacent to heterocysts may be due to disappearance of polyphosphates/ATPase activity in the vegetative cells adjoining heterocysts. This is inferred from the results obtained in normal filaments (control) where it was observed that fall in ATPase activity/polyphosphate content near the center of interheterocyst interval led to conversion of vegetative cells (present in that site) into spores.

In the filaments obtained from controls, initiation of spore differentiation took place subsequent to reduction in polysaccharides/polyphosphates/ATPase activity/TTC reducing activity/L-aspartate:2-oxoglutarate aminotransferase activity in the center of interheterocyst interval as a result of gradients established by heterocysts. This implies that under normal conditions heterocysts influence/regulate the formation of gradients which in turn control regularity in spacing of spore differentiation. Nichols et al. (1980)
suggested that spore formation in *Anabaena cylindrica* takes place when the repressive effect of some physiological aspect in vegetative cell is negatated by heterocysts. Findings of the present investigations with *Modularia spumigena* indicate that heterocysts regulate spore formation by creating gradients of electron transport activity/biochemical molecules leading to spore differentiation.
Phosphorus and amino acid composition
Exogenous supply of phosphate and nitrate plays an important role in the promotion of spore germination in blue-green algae (Reddy, 1976 and 1984 b). In the course of present studies, it was found that spores of Modularia spumigena germinated, to some extent, in the absence of phosphate or nitrate in medium. Since a considerable proportion of spores of Anabaena fertilissima and Anabaenopsis arnoldii could germinate in medium deficient in phosphate or nitrate, it was suggested that the spores contain endogenous reserves of these compounds (Reddy, 1984 b).

In the present study, analysis were performed to determine the phosphorus and amino acid composition of spores of Modularia spumigena. Matured spores (60 d old) were purified on density gradients and then used for analysis of phosphorus and amino acids.

A. PHOSPHORUS

Spores of Modularia spumigena which were formed in phosphate deficient medium and different light conditions were found to respond differently when they were allowed to germinate in medium with/without phosphate. Hence, investigations were performed to estimate the amount of phosphorus present in spores which were obtained from the cultures of Modularia spumigena grown in the presence of different qualities of light and medium containing/
devoid of phosphate.

Details of the procedure employed for the estimation of total phosphorus is provided in the section dealing with material and methods of investigations (Method IX).

Results (Ref. Table 4)

Phosphorus content of the spores formed in phosphate containing medium was found to be highest while the spores obtained from medium devoid of phosphate showed only trace amount of phosphate in them. Further, it was also found that phosphorus content of spores formed in white light was highest and it was followed by the spores formed in red, green and blue lights.

Discussion

From the results it is evident that the accumulation of phosphorus in spores of Modularia spumigena is dependent upon the presence or absence of phosphate in medium and quality of light in which the organism was grown. Depletion of phosphate in medium though hastened sporulation (see Fig. 6) it resulted in less accumulation of phosphorus in spores. Similarly, although red light slightly hastened the process of sporulation (see Fig. 2), it led to decreased accumulation of phosphorus compared to that in spores formed in white light. More accumulation of phosphorus
in spores obtained from the cultures grown in white light may be due to better growth of the organism in white light as compared to the growth in other lights.

B. AMINO ACIDS

Amino acids serve as nitrogen reserves in spores of blue-green algae. Yamamoto (1976) reported amino acid composition of vegetative cells and spores of Anabaena cylindrica. In the present investigation, studies were performed to ascertain amino acid composition of vegetative cells (4 d old), sporulating filaments (10 d old) and matured spores (60 d old) of Monularia spumigena.

Details of the paper chromatographic method employed in the present study are given in the section dealing with material and methods of investigations (Method X).

Result (Ref. Fig. 40 and Table 5).

Chromatographic separation of amino acids indicated the presence of arginine, aspartic acid, glutamic acid, alanine and phenylalanine in vegetative cells, sporulating filaments and matured spores. Further, an unknown amino acid was also common to all three materials. On the contrary, an unknown amino acid was found to be specific to vegetative cells while lysine was detected only in
sporulating filaments and in matured spores.

Discussion

In the present investigation, only a total of eight amino acids could be detected in the algal materials; probably because of one dimensional paper chromatographic method adopted in this study. Nevertheless, some differences in amino acid composition could be detected among vegetative cells, sporulating filaments and spores. In Anabaena cylindrica, lysine was found to be in trace amounts in vegetative cells as well as in spores (Yamamoto, 1976). In Modularia spumigena, detection of lysine in sporulating filaments but not in vegetative cells indicate that its content increased during spore formation. On the other hand, an unknown amino acid which was present in vegetative cells disappeared when the organism entered into sporulation stage. In spores of Anabaena cylindrica, compared to vegetative cells, contents of threonine, serine, glutamate, glycine, alanine, valine, isoleucine, leucine, tyrosine and phenylalanine decreased while proline content increased. These results obtained with Anabaena cylindrica (Yamamoto, 1976) and Modularia spumigena indicate that both qualitative as well as quantitative changes in amino acid composition occurs during spore formation in blue-green algae.
Very little information is available about the factors that induce germination of the spores in blue-green algae, although much is known in the case of bacteria.

Rodalaria spumigena, isolated from rice fields of Assam, India, is found to be an excellent material as it produces spores extensively within a short time in liquid medium. Further, almost all the vegetative cells of this alga are converted into spores (Fig. 1 c) and they can be separated without any drastic physical or chemical treatment. Therefore, the investigations on germination have been conducted with the purified spores (Fig. 1 d) which were collected from liquid medium.

The germination studies were done on nutrient agar plates as it was easy to follow the germination stages. In all following experiments, only fully matured spores (60 d old) were used.

Process of germination

When spores were plated on HG% medium, they turned green, became enlarged (Fig. 41 a), and enlargement was accompanied by the appearance of cytoplasmic granules in them. This stage was generally over by 24 h. In the next stage, spore coats ruptured (Fig. 41 b-d) and germlings, which were blue-green in colour and 1 cell in length,
emerged out (Fig. 41e). The bursting of spore wall and emergence of germling was considered as the criterion for determining the event of germination. By 96 h, germlings attained a maximum length of about 20 cells and were always straight (Fig. 41g-i). Simultaneously, heterocysts appeared in germlings, even in the presence of nitrate in growth medium. Initiation of sporulation was observed generally between 7 to 8 days in germlings.

Under certain conditions, such as when the organism was grown in medium containing ammonium salts, in situ germination (Fig. 41j,k) was observed. During such a process, cell division occurred inside spore wall and germlings which emerged were generally about 2 to 3 cells in length (Fig. 41j,k).

Sequence of germination

In complete BGZ medium, sequence of germination in a completely sporulated filament followed a particular pattern. Germination always commenced first in the spores which were located away from heterocysts (i.e., near the mid-point of filament between heterocysts) and later germination gradually took place in the spores present adjacent to heterocysts. In other words, a gradient of germination is established from near mid-point of sporulated filament towards heterocysts.
A. EFFECT OF ULTRAVIOLET (UV) IRRADIATION ON GERMINATION

Wu et al. (1967), Van Baalen (1968), Werbin and Rupert (1968) and Asato and Palsome (1969) established the presence of very strong photoreactiviting system in a number of blue-green algae. Srivastava and Kumar (1969) showed photoreversal of toxicity and mutagenicity of ultraviolet irradiated culture medium to spores of Anabaena doliolum. Comparative experimental studies on ultraviolet lethality and spore survival of Anabaena doliolum and Fischerella muscicola (Kumar, 1970), and Anabaena fertilissima and Anabaenopsis arnoldii (Reddy, 1976) were made. The effects of ultraviolet irradiation on spores of different variants of Fischerella muscicola (Singh and Singh, 1972b; Singh, 1975), and Anabaena vaginicola (Rai and Pandey, 1981) were investigated. In the present investigation, effect of ultraviolet irradiation on germination of spores of Modularia spumigena was studied.

Spores obtained from complete medium and normal growth conditions were employed in the investigation. Details of experimental procedures adopted for this study are given in the section dealing with methods of investigations (Methods IIA:a, IV and V).

Results (Ref. Fig. 42)

Compared to germination of spores in control,
ultraviolet irradiated spores exhibited a lag of 24 to 96 h, depending on ultraviolet dose, in germination (Fig. 42A).

At the end of incubation (i.e., 168 h), it was found that spores irradiated for 5 min showed a stimulation in germination (Fig. 42A:a) by about 8% as compared to control (Fig. 42B). On the other hand, spores irradiated for 10 to 20 min exhibited similar percentage of germination as that of in control while spores exposed to ultraviolet beyond 20 min showed lowering of percentage of germination. Survival of spores were not observed beyond 60 min of irradiation.

It was found that survival percentage of spores at 168 h after exposing them to ultraviolet of different doses exhibited two exponentially declining phases (Fig. 42B). The two phases were between 5 to 10 min and 20 to 60 min.

Sequence of germination

Both in control and ultraviolet treated sporulated filaments, it was found that spores which were midway between heterocysts germinated first and germination of spores gradually progressed towards heterocysts (Fig. 42A:b).
Discussion

Ultraviolet radiations have been used extensively to study the genetics of blue-green algae. Kumar (1963) isolated a resistant ultraviolet variant of *Anacystis nidulans* (Myer's strain) which exhibited the involvement of genetic elements in the control of ultraviolet lethality. It is evident from the present study that the spores of *Modularia spumigena* survived ultraviolet irradiation upto 20 min without showing a decrease in percentage germination. In case of *Anabaena fertilissima* (Reddy, 1976) and *Anabaena vaginicola* (Rai and Pandey, 1981), spores survived upto 1 min of ultraviolet irradiation without showing decline in percentage of germination compared to respective controls. On the other hand, spores of *Anabaenopsis arnoldii* survived ultraviolet dose upto 20 min (Reddy, 1976). Kumar (1970) found that the sensitivity to ultraviolet light in the spores of *Anabaena dolioiulm* and *Fischerella muscicola* was variable and suggested the presence of "some factors" which may ensure better survival of *Fischerella muscicola* under mutagenic environment. Singh and Singh (1972b) reported that the spores of two strains of *Fischerella muscicola* differ in their sensitivity to ultraviolet irradiation and suggested that the differences in the gene controlled repair phenomena may account for such variations. They also speculated that the decrease in production of thymine
containing photoproducts may be responsible for high resistance to ultraviolet irradiation in spores of one of the strains of *Fischerella muscicola*, basing their argument on existence of such a situation in the spores of a bacterium, *Bacillus megaterium* (Stafford and Donnellan, 1968).

Spores of *Modularia spumigena* have shown two exponentially declining phases between 5 to 10 min and 20 to 60 min of ultraviolet treatment. Such a trend in survival of spores may be due to existence of heterogeneous populations (two types) of spores.

An enhancement in the percentage of germination in *Anabaena doliolum* (Dikshit, 1974) and *Anabaenopsis arnoldii* (Reddy, 1976) was observed when the spores were irradiated with low doses of ultraviolet light. Similarly, an increase in the percentage germination (due to stimulation), was observed in the spores of *Modularia spumigena* irradiated for 5 min, though the germlings were found to be unhealthy than in control.

**B. EFFECT OF QUALITY OF VISIBLE LIGHT ON GERMINATION**

Harder (1917a) reported that spore germination in *Nostoc, Anabaena* and *Cylindrospermum* sp occurs only in light, but if sucrose is supplied it can take place in dark as well. It was shown that light controlled activation
of all physiological processes involved in blue-green algal spore germination is red light dependent (Reddy et al., 1975; Yamamoto, 1976) and can take place even in dark when stimulated by brief spells of red illumination, suggesting the non-photosynthetic light reaction (Reddy, 1976 and 1978).

So far, the information available with regard to the effects of quality of light is only on the ability of the spores formed in white light to germinate in different wavelengths of light (Harder, 1918; Kaushik and Kumar, 1970; Reddy et al., 1975; Yamamoto, 1976; Braune, 1979; Reddy and Talpasayi, 1981; Pandey and Talpasayi, 1981; Bai et al., 1985). Further, the studies which have been conducted by earlier workers also show that the spores formed in the media containing phosphate were always employed in the studies pertaining to assess the role of different qualities of light in germination. During the course of the present studies we have found that in Modularia spumigera, different spectral bands of light as well as the presence or absence of phosphate in the medium influence sporulation to different degrees (see page 89). However, no comparative studies have been conducted to investigate the germination responses of such spores (i.e., spores which are formed in different light conditions and in presence or absence
of phosphate) to various types of illuminations to enable
to understand their physiological status. Hence, studies
were undertaken to investigate the effects of different
spectral bands of light on germination of the spores formed
in the medium with/without phosphate as well as in different
light conditions.

The germination experiments were conducted in
HGZ medium containing phosphate and in presence of white,
red, green and blue lights. The observations were taken
at the end of 168 h. The method of study is presented
in the section dealing with material and methods of investi­
gations (Methods II A: b, IV and V).

Spores obtained from the cultures grown in the
presence of phosphate, and white, red, green or blue lights
are referred to in the text as 'spores: (+P: W)', 'spores: (+P: R)',
'spores: (+P: G)' or 'spores: (+P: B)', respectively, while
the spores collected from the cultures grown in the presence
of white light and the medium lacking phosphate are referred
to as 'spores: (-P: W)'.

Results (Ref. Tables 6 and 7)

Spores did not germinate in dark. In all types
of illuminations, both in the presence or absence of phosphate
in the medium, initiation of germination was accompanied
by similar morphological changes in spores and they are reported sequentially. The process of germination began with the greening of spores. They became enlarged accompanied by the appearance of cytoplasmic granules. The final event of germination process was the emergence of the germlings by breaking open the spore coats.

Germination responses of spores formed in different qualities of light to similar or different wavelengths of light: In white light, the percentages of germination of 'spores: (+P:W)', 'spores:(+P:R)', 'spores:(+P:G)' and 'spores:(+P:B)' were about 71, 89, 59 and 67, respectively, while in red illumination, their germination was about 73, 43, 24 and 46% (Table 6). In green and blue illuminations, the germination of all categories of spores was much lower compared to that in white as well as in red lights. However, it was found that about 9 and 17% of 'spores:(+P:G)' and 'spores:(+P:B)' germinated in green and blue lights, respectively, while only 3 to 5% of 'spores:(+P:W)' and 'spores: (+P:R)' germinated in similar green and blue illuminations.

Germination responses of spores formed in the medium with/without phosphate to different wavelengths of light: In white light, the percentages of germination of 'spores:(+P:W)' and 'spores:(-P:W)' were about 71 and 58, respectively, whereas in red light, the germination percentages were
about 73 and 13 (Table 7). In green and blue spectral bands of light, only about 1 to 4% of the 'spores: (+P:W)' and 'spores: (-P:W)' germinated.

Discussion

The initial processes of germination in all categories of spores were found to be similar while the ability to complete all the processes of germination differed from one category to another depending on the conditions of light incubation. This observation suggests that any wavelength of light tested is able to promote the initiation processes of germination while the processes beyond initiation stage seem to be governed by the quality of illumination.

The spores of <i>Modularia spumigena</i> germinated to different extents in all qualities of light but it was generally better in white light than in red, green and blue lights. Kaushik and Kumar (1970) studied the stimulation of spore germination of <i>Anabaena doliolum</i> in different qualities of light and reported that germination occurs in white, red, green and blue lights. They also reported that the different spectral bands behaved similarly in promoting germination. Like in <i>Anabaena doliolum</i>, in <i>Anabaena variabilis</i> the germination was found to occur in all wavelengths of light from 404 to 702 nm, but the most effective
spectral range for highest germination was between 620 to 630 nm (Braune, 1979). However, in the case of spores of Anabaena fertilissima and Anabaenopsis arnoldii (Reddy, 1976 and 1984c), and Modularia spumigena (Pandey and Talpasayi's strain isolated from rice fields of Banaras; Pandey and Talpasayi, 1981), different spectral bands behaved differently in promoting germination. It was reported that in Anabaena fertilissima and Anabaenopsis arnoldii, spores germinated only in white and red lights but failed to germinate in green and blue lights (Reddy, 1976 and 1984c) while the spores of Modularia spumigena (Pandey and Talpasayi's strain) germinated in white, red and blue lights but not in green (Pandey and Talpasayi, 1981). Comparing the results of the present study with those findings obtained with other blue-green algae, it is apparent that the spores of Modularia spumigena (strain of the present study) resemble the spores of Anabaena doliolum and Anabaena variabilis in their ability to germinate in different qualities of light and Anabaena variabilis, Anabaena fertilissima, Anabaenopsis arnoldii and Modularia spumigena (Pandey and Talpasayi's strain) in their capability to germinate most effectively in white/red illuminations. Further, the ability of spores of Modularia spumigena (strain of the present study) to germinate better in white light than in other qualities of illumination suggest
that a combination of different spectral bands of light is essential for better germination.

No information is available pertaining to the influence of different wavelengths of visible light on the germination of spores formed in similar or different qualities of illumination. In the present study with Modularia spumigena, it was found that the spores formed in white and red lights showed a poor germination in green and blue lights while the spores formed in green and blue lights germinated better in the respective illuminations. This finding suggests that the spores formed in green and blue lights acquired a physiological/biochemical capability which confer the ability to germinate better in similar light conditions which promoted their differentiation.

In Modularia spumigena, spores formed in the medium containing phosphate (i.e., 'spores:(+P;W)') had more phosphorus reserves than the spores formed in the medium lacking phosphate 'i.e., 'spores:(-P;W)') (see page 144). In the present study, it was found that the 'spores:(+P;W)' germinated better (in almost all light conditions tested) than the 'spores:(-P;W)' when the both categories of spores were allowed to germinate in the medium containing phosphate. These results indicate that inspite of the availability
of phosphate in the external medium, the spores which have more endogeneous phosphorus reserves showed better germination response when exposed to light compared to the spores in which the phosphorus reserves are less or nil. Based on these results it may be suggested that the endogenous reserves of phosphorus play an important role in regulating spore germination than the phosphorus supplied exogenously. Alternatively, it may also be possible that a considerable proportion of the spores formed in the medium devoid of phosphate might have been non-viable and hence, failed to germinate even in the presence of phosphate. Reddy (1984b) working with the spores of Anabaena fertilissima and Anabaenopsis arnoldii observed that a considerable proportion of the spores formed in normal medium failed to germinate when inoculated in the medium lacking phosphate and suggested that the endogenous phosphorus reserves in majority of spores may not be sufficient to support the completion of all the processes of germination, thereby implying the necessity of exogenous supply of phosphate to promote germination.

C. EFFECT OF TRACE ELEMENTS ON GERMINATION

Presence of microelements in the medium is essential for the growth of blue-green algae. Their absence from the medium affect different physiological and biochemical
processes in these organisms (see Fogg et al., 1973; Krogmann, 1973; Bothe, 1983). It seems that no study has been conducted so far to investigate the effects of trace elements on spore germination in blue-green algae though information is available in case of bacteria. Hence, a study was undertaken to investigate the effects of different micronutrients on spore germination in Nodularia spumigena.

Spores obtained from the cultures grown in complete BGZ medium were used. Details pertaining to the method of study are given in the section dealing with the material and methods of investigations (Methods II B:a i-vii, IV and V).

Results (Ref. Fig. 43)

The spores incubated in the medium lacking boron, zinc and copper showed a slight stimulation in germination in the initial period upto 72-96 h as compared to control (Fig. 43). However, at the end of 168 h, the germination percentage in the medium lacking one microelement at a time was lower than that in control which was 74% (Fig. 43a). At 168 h, the germination of spores was lowest in the medium lacking all trace elements compared to the germination in the medium devoid of one microelement at a time (Fig. 43 a). When individual trace elements were
omitted from the medium it was found that at 168 h, the germination was lowest in the medium lacking cobalt (48%) followed by in the medium in which copper (52%), molybdenum (52%), manganese (55%) or zinc (60%) were excluded.

Sequence of germination: Similar to control, germination sequence in the medium lacking all trace elements as well as in the medium where only manganese or molybdenum or cobalt was omitted, the germination of spores started near the mid-point of the filament between heterocysts and progressed towards heterocysts (Fig. 43 b). On the other hand, in the medium lacking either boron or zinc or copper, the spores adjacent to heterocysts started germinating first and the germination progressed towards the mid-point of filament between heterocysts.

Discussion

Different cations and anions have been reported to influence the growth of blue-green algae (Holm-Hansen, 1968). However, no studies concerned with the role of micronutrients on the germination of spores of these organisms have been made. On the other hand, the influence of microelements on the spore germination in bacteria has been reported (see Gould, 1969).

Germination of spores of *Modularia spumigena*
(though reduced to a certain degree in the absence of trace elements) in the medium devoid of trace elements suggest that enough quantities of these elements are present in the spores and they are sufficient to support germination in the absence of exogenous supply of trace elements. In fact, accumulation of high concentrations of these elements have been reported in bacterial spores (see Murrell, 1969).

A slight stimulation of germination, up to 72-96 h, in the medium lacking boron, zinc or copper indicate that sufficient amounts of these elements may already be present in the spores and the additional input of them in the medium may lead to a slight suppression of germination in the initial stages. Compared to control, lowering of percentage of germination, at 168 h, in the medium lacking either all the trace elements or one trace element at a time, indicate that their exogenous supply is essential, to some extent for the germination of the spores of Modularia spumigena at later stages. Further, different degrees of inhibition of germination upon the removal of boron, cobalt, copper, molybdenum, manganese or zinc from the medium indicate their relative importance in the germination processes. It also suggests that the presence of these elements is essential for different biological processes which regulate germination. It is well established that
elements like manganese, zinc, cobalt, molybdenum, copper etc. are essential for many enzyme activities such as carboxypeptidase, phosphotransferases, arginase, cytochrome oxidase, nitrate reductase etc (see Lehninger, 1976) which are all shown to take part in the metabolic processes of bacterial spores during germination (see Gould, 1969). In fact, it was shown that in *Anabaena* SP 310, the protease activity in germinating spores increased by about 1.5 to 2 fold in the presence of copper ions (Reddy and Sarada, 1985).

The studies on the sequence of germination of spores in the medium devoid of either boron or zinc or copper showed that spore germination started near heterocyst and progressed towards the mid-point of filament between heterocysts. On the contrary, sequence of germination of spores in the medium devoid of manganese, molybdenum or copper followed exactly an opposite pattern and under these conditions germination started in the middle of the filament and progressed towards heterocysts. These findings suggest that the spores formed adjacent to heterocysts contain sufficient amounts of boron, zinc and copper endogenous reserves while the spores formed away from heterocysts seem to have enough quantities of manganese, molybdenum and cobalt to support germination in the medium lacking these elements.
D. EFFECT OF NITRATE ON GERMINATION

Though much is known about the role of nutrients like nitrogen in different processes related to growth and metabolism including the nitrogen fixation in blue-green algae, the knowledge about their role in spore germination is limited. In Anabaena fertilissima and Anabaenopsis arnoldii, it was observed that exogenous supply of nitrate plays an important role in the promotion of germination of spores (Reddy, 1976 and 1984b). In the present study, investigations were carried out to assess the effects of nitrate deficiency in the medium on spore germination in Modularia spumigena.

In this study, spores obtained from the complete medium, i.e., the medium containing nitrate, were employed in the investigations on germination in the medium with/without nitrate. The method of study is given in the section dealing with material and methods of investigations (Methods II B:a viii, IV and V).

Results (Ref. Fig. 44)

It was observed that when the spores were plated on the medium both with (control) or without nitrate showed a conspicuous lag in germination up to 24 h (Fig. 44). At 168 h, the germination percentage was very much reduced
in the medium lacking nitrate (by about 39%) as compared to the percentage germination on complete medium (Fig. 44a).

Sequence of germination: In the medium with nitrate (control) spore germination initially started in the spores situated near mid-point of filament between heterocysts and it was gradually followed by the germination of the spores located near heterocysts (Fig. 44 b). On the contrary, the sequence of germination of spores in the medium devoid of nitrate commenced first in the spores located near to heterocysts and later spores present away from heterocysts gradually germinated.

Discussion

Compared to about 71% of germination in the medium having nitrate (control), only about 43% of the spores germinated in nitrate deficient medium. This type of differential behaviour of spores, in presence or absence of nitrate in the medium, may be due to the developmental differences. As such, in a population of spores of Modularia spumigena, there were differences in their sizes. Thus it may be suggested that there can also be certain intrinsic differences in the amounts of nitrogen reserve present in the spores. Since the spores located near to heterocysts in filaments exhibited an ability to germinate readily in the absence of exogenous supply of nitrate in the medium
than the spores which were situated away from heterocysts, it indicates that the spores which are present near to heterocysts contain more nitrogen reserves compared to the spores which are formed away from heterocysts.

Not much is known about the nature of nitrogen reserves in the blue-green algal spores. The electron microscopic studies of the spores of several blue-green algae showed the presence of cyanophycin granules in them (Ris and Singh, 1961; Clark and Jensen, 1969; Jensen and Clark, 1969; Rao, 1977). Cyanophycin granules were found to be a rich store of combined nitrogen (Simon, 1971). Chromatographic and histochemical investigations revealed that the spores of *Modularia spumigena* contain amino acids like alanine, arginine, aspartic acid, glutamic acid, lysine, phenylalanine and tryptophan (see Table 5). In the present study, the results pertaining to the spores which, in spite of having different amino acids in them, failed to germinate in the medium lacking nitrate indicate that the nitrogen reserves in these spores are either not fully utilized or insufficient for the completion of germination processes.

E. EFFECT OF PHOSPHATE ON GERMINATION

The importance of phosphorus in different processes related to growth and metabolism in blue-green algae is
well documented (see Healey, 1983). However, the information concerned with the role of this element in spore germination is limited to a few species of blue-green algae. Further, it is not known how the spores formed in different growth conditions respond to the presence/absence of phosphorus in the external medium.

In this study, the spores formed in the presence/absence of phosphate and in different light conditions were used. Spores obtained from the cultures grown in the presence of phosphate and in white, red, green or blue lights are referred to in the text as 'spores:(+P:W)', 'spores:(+P:R)', 'spores:(+P:G)' or 'spores:(+P:B)' respectively, while the spores collected from the cultures grown in the presence of white light and in the medium lacking phosphate are referred to as 'spores:(-P:W)'.

The germination experiments were conducted in HGZ medium with/without phosphate. The method of study is presented in the section dealing with material and methods of investigations (Methods IIB:a ix, IV and V).

Results (Ref. Table 8 and Fig. 45)

Germination of 'spores:(+P:W)' and 'spores:(-P:W)' in the medium with/without phosphate: Both 'spores:(+P:W)' and 'spores:(-P:W)' plated on agarized complete medium
exhibited a lag in germination up to 24 h while those inoculated on the medium devoid of phosphate germinated after a lag of 48 h (Fig. 45 A, B). In the absence of phosphate in the medium, at 168 h, the germination of 'spores:(+P:W)' fell to about 26% compared to 71% of germination in the medium containing phosphate (control) (Fig. 45 A:a). In case of 'spores:(-P:W)', about 48% germinated in the medium lacking phosphate compared to about 53% germination in the medium containing phosphate (control) (Fig. 45 B:c).

Germination of 'spores:(+P:W)', 'spores:(+P:R)', 'spores:(+P:G)' and 'spores:(+P:B)' in the medium with/without Phosphate: All these four categories of spores, germinated better in the medium containing phosphate than in the medium devoid of phosphate (Table 8). At 168 h, the percentage of germination of 'spores:(+P:W)', 'spores:(+P:R)', 'spores:(+P:G)' and 'spores:(+P:B)' were found to be about 71, 89, 59 and 67, respectively, in the medium with phosphate while in the medium without phosphate, germination percentages were about 22, 32, 29 and 62. Maximum percentage of germination was observed with 'spores:(+P:R)' (89%) in the medium containing phosphate while 'spores:(+P:B)' showed highest percentage of germination (62%) in the medium devoid of phosphate. Further, in the medium lacking phosphate, germination of 'spores:(+P:R)' and 'spores:(+P:B)' was better than the 'spores:(+P:W)'.
Sequence of spore germination: Sequence of germination of 'spores:(+P:W)' and 'spores:(-P:W)' was similar in a given combination (with regard to presence/absence of phosphate) of the medium. In phosphate containing medium, spores (both 'spores:(+P:W)' and 'spores:(-P:W)') which were formed away from heterocysts commenced germination first and it was gradually followed by the germination of spores which were produced near to heterocysts (Fig. 45A:b and 45B:d). On the contrary, in the medium lacking phosphate, the spores which were formed near to heterocysts germinated first whereas the spores which were located away from heterocysts gradually germinated later.

Discussion

It is not known as to how the absence of phosphate affects the germination of the spores of the alga. In the present investigation, it was found that only a certain proportion of 'spores:(+P:W)' and 'spores:(-P:W)' germinated in the medium devoid of phosphate. Further, in the medium deficient of phosphate, the spores formed adjacent to heterocysts germinated more efficiently than the spores which were located away from heterocysts. This type of differential behaviour of spores, under similar conditions, may be due to intrinsic differences in the amounts of phosphorus reserves present in spores thereby implying
more phosphorus reserves in spores which are produced near to heterocysts and relatively less phosphorus reserves in spores developed away from heterocysts. It can be mentioned here that the rate of respiration increases during the spore germination (Pay, 1969b) indicating greater utilization of reserve materials to meet the energy demand of developing germlings. Hence, it may be suggested that the spores which contain sufficient amounts of phosphorus reserve material to meet the needs of high metabolic activities during germination in the nutrient deficient conditions germinate better than the spores which contain less endogenous reserves. Further, the failure of some spores to germinate in phosphate limited conditions may also be due to the inability to utilize phosphorus reserves present in them.

Very limited information is available about the nature of phosphorus reserves in blue-green algal spores. Histochemical analysis showed that the matured spores of *Modularia spumigena* lacked polyphosphate materials (see page 120). In the present investigations, the results pertaining to the ability of some of the spores, which lack polyphosphate, to germinate in phosphate deficient medium indicate the phosphorus reserves in these spores are present in a form other than polyphosphate material.
Analysis of phosphate content of spores has showed that 'spores: (+P:W)' contain more phosphorus content than the 'spores: (-P:W)' (Table 4). In spite of having very low content of phosphorus 'spores: (-P:W)' germinated better about 48% germination than the 'spores: (+P:W)' (about 26% germination), when they were allowed to germinate in the medium lacking phosphate. The reason for such an efficient germination of the 'spores: (-P:W)' than the 'spores: (+P:W)' in the medium lacking phosphate is not clear.

A comparison of the germination responses of spores formed in different qualities of light reveals that 'spores: (+P:R)' and 'spores: (+P:B)' germinated better than 'spores: (+P:W)' in the medium devoid of phosphate (Table 8). Analysis of phosphorus content of spores indicated that 'spores: (+P:W)' contained highest quantity of phosphorus and it was followed by 'spores: (+P:R)', 'spores: (+P:G)' and 'spores: (+P:B)' (Table 4). In spite of having a low content of phosphorus, 'spores: (+P:R)' and 'spores: (+P:B)' germinated better than 'spores: (+P:W)' in the medium devoid of phosphate. This observation suggests that 'some factor(s), other than phosphorus reserves, responsible for efficient germination is accumulated in 'spores: (+P:R)' and 'spores: (+P:B)' when they are formed in the presence of photosynthetically
efficient red and blue lights, and that 'factor(s)' probably triggers good germination in phosphate deficient conditions. Further work can only unfold the nature of the 'factor(s)'.

F. EFFECT OF pH ON GERMINATION

Blue-green algae have a wide distribution. Fogg et al. (1973) stated that hydrogen ion concentration is an important factor which directly or indirectly affects the distribution and abundance of blue-green algae. Most of the members of cyanophyceae grow in the pH range 7 to 9 (Gerloff et al., 1950; Kartz and Myers, 1955 and Granhall, 1970).

Reddy (1976 and 1984a) reported that the range of pH for maximum germination is wider than the limits for good vegetative growth in Anabaena fertilissima and Anabaenopsis arnoldii. On the contrary, in Anabaena cylindrica, the pH range for spore germination as well as vegetative growth was found to be same (Yamamoto, 1976). Above studies pertaining to spore germination in different pH conditions were done in medium containing nitrate in case of Anabaena fertilissima and Anabaenopsis arnoldii and in medium devoid of nitrate in case of Anabaena cylindrica. In the present study, investigations were undertaken to assess the effects of pH on spore germination in Modularia spumigena, both in presence or absence of nitrate or phosphate in medium.
Further, a comparative study was also conducted to assess the influence of different qualities of light on germination abilities of spores in complete medium maintained at different pH conditions.

Experiments were conducted with the spores obtained from the cultures grown in the presence of complete medium and white light. Details of methods employed in this study are presented in the section dealing with materials and methods of investigations (IIB:b, IV and V).

Results (Ref. Table 9 and Fig. 46)

Germination responses of spores in complete medium and medium without nitrate or phosphate maintained at different pH conditions: In complete medium, germination was observed at all pH conditions ranging from 5 to 12 while in the medium lacking nitrate or phosphate, spores did not germinate at pH 12 (Fig. 46). Further, at pH 8.5 (control), spores in complete medium exhibited a lag in germination up to 24 h whereas in all other pH conditions lag in germination was generally observed up to 48 h/72 h (Fig. 46A). In medium devoid of nitrate, spores exhibited a lag in germination up to 48 h and 72 h at pH conditions 11 and 5, respectively (Fig. 46B). In other pH conditions including at pH 8.5 (control) lag in germination was observed to be only up to 24 h. On the other hand, in medium lacking
phosphate, lag in germination, in all pH conditions including at pH 8.5 (control), was 48 h (Fig. 46C).

In alkaline pH conditions from 9 to 11, a faster rate of spore germination was observed till 120 h in complete medium (Fig. 46A). However, such an enhancement in the rate of germination was not observed in medium lacking nitrate or phosphate (Fig. 46B and C).

In complete medium, at 168 h, highest percentage of germination (about 100%) was observed in pH range 9 to 11 followed by germination in pH range 7 to 8.5 (about 71%) (Fig. 46A:a). The percentages of germination at pH 5, 6 and 12 were found to be about 28, 54 and 46, respectively. In case of medium lacking nitrate, about 64% of the spores germinated at pH 9 which was followed by about 42 - 43% of germination in pH range 6 to 8.5 (Fig. 46 B:c). On the other hand, in medium devoid of phosphate, about 31% of the spores germinated at pH 8 which was followed by 21 - 26% of germination at pH 6, 7, 8.5, 9, 10 and 11 (Fig. 46 C:e). In this medium, only about 17% of spores germinated at pH 5.

Among all pH conditions tested, percentage germination was higher in complete medium compared to medium lacking nitrate or phosphate excepting at pH 5 where more number of spores (about 42%) germinated in medium devoid
of nitrate (Fig. 46; compare insets a, c, e).

Germination responses of spores in complete medium maintained at different pH conditions and exposed to various qualities of light: In all pH and light conditions, spores germinated though to a varied degrees (Table 9). In all pH conditions, compared to white and red lights, germination was low (1 to 10%) when spores were allowed to germinate in green and blue lights. Percentage germination of spores was higher in red than in white light at pH 5, 6 and 12. Red light generally supported highest percentage of germination (100%) in highly alkaline pH conditions (i.e., pH 10 and 11) while in white light, highest percentage of germination (100%) was observed between pH 9 and 11.

Sequence of spore germination: In all pH conditions, sequence of spore germination was similar for a particular combination of medium. In complete medium, germination in a chain of spores (sporulated filament), started with the spores which are located away from heterocysts and germination proceeded progressively towards heterocysts. On the contrary, in medium lacking either nitrate or phosphate, the spores adjacent to heterocysts germinated earlier than the spores which are away from heterocysts.

Discussion

A limited information which is available on spore
germination in varied pH conditions were all done in complete
growth media and in normal light conditions (Reddy, 1976
and 1984a, Yamamoto, 1976; Rai and Pandey, 1981). Results
of the present study indicate that germination responses
of spores to varied pH conditions depends, to some extent,
on presence of nutrients such as nitrate and phosphate in
medium as well as quality of light to which they are exposed.

In complete medium and under white light, germination
of spores of Nodularia spumigena (which grows better at
pH 8.5) occurred between pH 5 - 12, the most rapid being
between pH 9 to 11. These observations clearly indicate
that different pH conditions are required for healthy growth
of vegetative filaments and for rapid germination of spores.
Such a phenomenon of spore germination was also observed
in case of Anabaena fertilissima and Anabaenopsis arnoldii
(Reddy, 1976 and 1984a), and Anabaena vaginicola (Rai and
Pandey, 1981). Reasons for such a differential requirement
for growth and spore germination are not clearly understood.
Further, similar to germination of spores in Nodularia
spumigena, germination in Anabaena fertilissima and Anabaenopsis
arnoldii (Reddy, 1976 and 1984a), Anabaena cylindrica
(Yamamoto, 1976) and Anabaena vaginicola (Rai and Pandey,
1981) occurred in a wide range of pH conditions.

In the present investigation, it was found that
the pH conditions responsible for stimulation of germination varied depending upon the nature of medium and conditions of light to which the spores are exposed. Compared to germination at pH 8.5 in respective media (controls), percentage of germination increased by about 41% in pH range 9 to 11 in complete medium while it increased to 48% at pH 9 in medium devoid of nitrate and to about 24% at pH 8 in medium lacking phosphate (Fig. 46 D - F). In other words, optimum pH conditions for maximum germination shifted down from 9 - 11 in complete medium to 9 in medium lacking nitrate and to 8 in medium without phosphate. Further, it was also observed that the range of optimum pH conditions for highest germination (100%) decreased when illumination is changed from white to red. From these observations it is evident that different pH conditions stimulate highest germination in response to presence or absence of nitrate/phosphate in medium and different light conditions to which spores are exposed. It may be suggested that such a stimulation of germination at varied pH conditions in different combinations of medium or light sources may be due to activation of different germination systems, existing in spores, in different conditions.

G. EFFECT OF AMINO ACIDS ON GERMINATION

Amino acids are building blocks of proteins. Blue-
green algae being photoautotrophic organisms can synthesize all amino acids necessary for their growth (Carr, 1973). Studies were conducted to assess the influence of externally supplied amino acids on growth and amino acid biosynthesis in *Anabaena variabilis* (Hood and Carr, 1971) and *Anacystis nidulans* (Weber and Boch, 1969). However, information on the influence of exogenously supplied amino acids on spore germination is scanty, and probably the only report on this topic is on germination in *Anabaena cylindrica* (Yamamoto, 1976). Hence, in the present investigation, experiments were conducted to study the effects of exogenously supplied amino acids on germination of spores of *Modularia spumigena* both in presence and absence of nitrate in medium.

Spores obtained from cultures grown in complete medium were employed in the present investigations. The amino acids used in these studies were L-arginine, L-aspartic acid, L-isoleucine, L-leucine, L-phenylalanine and L-tryptophan. Methods of study are given in the section dealing with material and methods of investigations (Methods IIB:d, IV and V).

**Results** (Ref. Figs. 47 to 52)

In the initial period upto 48 h, compared to germination in medium with nitrate, generally a slight enhancement in percentage of germination was observed in medium devoid
of nitrate (Figs. 47A, 48A, 49A, 50A, 51A and 52A). In medium lacking nitrate, increase in percentage of germination up to 48 h, was further stimulated when L-arginine, L-aspartic acid, L-isoleucine, L-leucine, L-phenylalanine or L-tryptophan was included in medium. On the contrary, in the same period, a marginal inhibition was observed when these amino acids were supplied in medium containing nitrate (Figs. 47A, 48A, 49A, 50A, 51A and 52A).

At 168 h, germination was more in nitrate containing medium compared to that in medium lacking nitrate (Figs. 47A:a, 48A:a, 49A:a, 50A:a, 51A:a and 52A:a). Inclusion of any one of the amino acids in medium lacking nitrate resulted in percent increase of germination when added up to a concentration of 0.1 μM (L-aspartic acid and L-phenylalanine) or 1 μM (L-arginine, L-isoleucine, L-leucine and L-tryptophan) (Figs. 47B, 48B, 49B, 50B, 51B and 52B). But addition of any of the amino acids in medium having nitrate brought about decrease in percent germination of spores. Inclusion of amino acids, other than arginine, in higher concentration (10 μM) completely inhibited germination in medium having nitrate. On the other hand, addition of similar high concentrations of amino acids in medium devoid of nitrate though, to some extent, resulted in percent decrease in germination, they did not completely inhibit germination of spores as in case of medium with nitrate.
Sequence of germination: It was observed that in medium with nitrate, irrespective of the presence or absence of amino acids, germination started in a sporulated filament, first in the spores located away from heterocysts, and later the spores situated near to heterocysts gradually germinated (Figs. 47A:b, 48A:b, 49A:b, 50A:b, 51A:b and 52A:b). On the other hand, in medium devoid of nitrate, regardless of the presence or absence of the amino acids, the spores present near to heterocysts started germinating first and germination gradually progressed towards the mid-point of filament between heterocysts. These findings indicate that only the presence or absence of nitrate in medium has an influence on sequence of spore germination and it is not altered by the presence of the amino acids like L-arginine, L-aspartic acid, L-isoleucine, L-leucine, L-phenylalanine or L-tryptophan in medium.

Discussion

The results of the present study showed that addition of lower concentrations (0.1 and 0.1 μM) of L-arginine, L-aspartic acid, L-isoleucine, L-leucine, L-phenylalanine or L-tryptophan in medium lacking nitrate stimulated germination whereas their inclusion in medium containing nitrate resulted in decreased percentage of spore germination (Figs. 47B, 48B, 49B, 50B, 51B and 52B). On the other hand, exogenous
supply of high concentrations (10 μM) of individual amino acids was found to be inhibitory to germination of spores both in nitrate containing and nitrate lacking medium, though the inhibition was much less in medium lacking nitrate. Yamamoto (1976) using very high concentrations of amino acids in medium lacking nitrate observed that L-phenylalanine (302.7 μM) and L-isoleucine (381.2 μM) inhibited spore germination in *Anabaena cylindrica* while aspartic acid (375.7 μM and 751.3 μM) stimulated it. It was also reported that higher concentration of L-phenylalanine (605.5 μM) is more inhibitory to spore germination than lower concentration (302.7 μM). Influence of arginine, leucine and tryptophan on germination of spores of *Anabaena cylindrica* was not studied. However, it seems that spores of *Nodularia spumigena* are more sensitive to the presence of amino acids in external medium than spores of *Anabaena cylindrica*.

Amino acid content of spores of *Anabaena cylindrica* was reported to be about 20% of that present in vegetative cells (Yamamoto, 1976). Histochemical and chromatographic analysis showed that arginine, aspartic acid, phenylalanine and tryptophan together with alanine, lysine and glutamic acid are present in spores of *Nodularia spumigena*, while isoleucine and leucine were not detected (see Table). Amino acids present in spores may be serving as nitrogen reserves and probably they are essential for different
metabolic processes during germination. However, it is not clear as to why exogenously supplied amino acids, particularly in nitrate containing medium, are inhibitory for germination. However, a probable reason for such an inhibition of germination may be diversion of ATP reserve of spores, which is otherwise needed for promoting germination processes, for uptake of amino acids against concentration gradient. This suggestion is based on the findings in *Nitzschia ovalis* (North and Stephens, 1972) and *Escherichia coli* (Berger, 1973) in which uptake of amino acids was observed to take place against gradient by expending ATP. Studies on the regulatory mechanism of amino acids in germination processes in blue-green algae are necessary to understand this phenomenon.

H. EFFECT OF GROWTH REGULATORS ON GERMINATION

Growth regulators influence different processes concerned with seed germination in higher plants (see Ovcharov, 1969; Khan, 1980) and removal of spore dormancy in a green alga *Hydrodictyon reticulatum* (Sussman and Halvorson, 1966). In blue-green algae, probably the only report on the influence of growth regulators, such as gibberellic acid and kinetin, on spore germination is in *Anabaena azollae* (Bai et al., 1981). In the present study, experiments were conducted to investigate the effects of growth regulators like
2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA), 6-furfurylaminopurine (kinetin) and gibberellic acid (GA₃) on spore germination in *Nodularia spumigena*.

In this study experiments were conducted on complete HGZ medium supplemented with one growth regulator at a time. Spores obtained from cultures grown in normal conditions were used. The methods of investigation are presented in the section dealing with material and methods (Methods IIB: e i-iv IV and V).

**Results** (Ref. Figs. 53 to 56)

Compared to control, kinetin and GA₃ stimulated germination, though slightly during first 24 h (Figs. 55A and 56A). However, concentrations of 2,4-D and IAA used in the present study did not stimulate germination upto 24 h and it was found to be same as in controls (Figs. 53A and 54A).

In comparison to control at 168 h, 0.025 and 0.05 µM of 2,4-D increased the percentage of germination while the concentration beyond 0.1 µM inhibited it (Fig. 53A:a). Similarly, inclusion of IAA also stimulated germination upto a concentration of 0.15 µM whereas 0.25 and 0.5 µM was found to be inhibitory for germination of spores (Fig.54A:a)
168 h, all the concentrations of kinetin and GA_3 used in the study increased the percentage of germination, though concentration beyond 0.15 μM lowered such an increase in germination to some extent (Figs. 55A:a and Fig. 56A:a).

A comparison of the figures 53B, 54B, 55B and 56B reveals that maximum percent increase in germination was with IAA up to a concentration of 0.15 μM and it is followed by GA_3, kinetin and 2,4-D. IAA, 0.5 μM, decreased germination by about 16% while a similar concentration of 2,4-D completely inhibited the germination (Figs. 53B and 54B).

Sequence of germination: Inclusion of growth regulators in medium did not influence sequence of germination. As in control, spore germination commenced near the mid-point of filament between heterocysts and it gradually progressed towards heterocysts (Figs. 53A:b, 54A:b, 55A:b and 56A:b).

Discussion

2,4-D which is generally used as a herbicide needs a due consideration to evaluate a possible impact of this chemical on the germination of spores of blue-green algae.

It was found that 2,4-D functions as growth stimulator of plants when it is supplied in lower concentration (see Leopold and Kriedemann, 1980). It has been shown that the pre-soaking of rice seeds in low concentrations of 2,4-D
increased seed germination (Aleshin and Erygin, 1957). In the present investigation with spores of *Modularia spumigena* it was found that 2,4-D upto a concentration of 0.05 μM stimulated germination while higher concentrations were inhibitory. Srivastava and Tiwari (1985) reported that low concentrations of 2,4-D increased protein and RNA synthesis while decreased DNA synthesis in *Anabaena doliolum*. However, the exact mode of action of 2,4-D could not be ascertained.

Spores of *Modularia spumigena* germinated faster and showed high percentage of germination when supplied with IAA upto a concentration of 0.15 μM. In higher plants also such stimulation of seed germination was observed with IAA treatment (Simakin, 1966).

Jann and Amen (1980) stated that auxins (such as 2,4-D and IAA) are critical factors in seed germination of higher plants because of their effects on cell elongation and structural components. It was also reported that they are involved in α-amylase production (Jann and Amen, 1980) which breaks down reserve carbohydrates. It is worthwhile to mention here that a high α-amylase activity was reported in the spore of *Anabaena sp* (Sarma et al., 1977). The stimulatory effect of IAA is related to the increased conversion of reserve nutritional materials into mobile compounds.
with a more pronounced synthesis of RNA in the germinating seeds of higher plants (Ovcharov, 1969).

Spores of *Modularia spumigena* responded to the application of kinetin in the medium and germination was found to be higher than in control. Similarly, spores of *Anabaena azollae* also showed an enhancement in germination in presence of kinetin (Bai et al., 1981). Though there are no reports with regard to the influence of kinetin on metabolism of blue-green algal spores, much information is available on the seeds of higher plants. It was shown that cytokinins participate in the biosynthesis of protein, chlorophyll and other vitally important compounds in the germinating seeds (Kulaeva and Klyachko, 1965). Recent studies indicate that cytokinins regulate nuclear translation and protein synthesis in seed germination (see Thomas, 1980).

Like the effect of kinetin, GA₃ also enhanced spore germination in *Modularia spumigena*. Bai et al. (1981) also reported that GA₃ stimulated spore germination in *Anabaena azollae*. In higher plants, the stimulatory effects of applied gibberellin on the germination of both dormant and non-dormant seeds has been widely reported (see Lang, 1970; Villiers, 1972). Indeed gibberellins regulate a wide variety of mechanisms in seed germination; DNA and RNA synthesis
and many enzymatic activities such as of acid phosphatase, α-amylase, hydrolases, iso-peroxidases, etc. (see Khan, 1980).

A survey of the literature pertaining to the effects of growth regulators showed that these compounds stimulate RNA synthesis in the germinating seeds of higher plants, and thereby bringing about more protein synthesis in them. It is reported in case of the germinating spores of blue-green algae such as Anabaena doliolum (Singh and Sunita, 1974), Anabaena sp (Grilli Caiola and Favali, 1982a, b) and Nostoc 7524 (Sutherland et al., 1985) that RNA synthesis starts very early during germination. It is possible that different growth regulators tested may stimulate RNA synthesis in germinating spores thereby leading to protein synthesis which was shown to be critical for initiation of germination (Rai, 1980).

I. EFFECT OF UNCOUPLER AND, ENERGY AND ELECTRON TRANSFER INHIBITORS ON GERMINATION

2,4-dinitrophenol (DNP; and uncoupler), N-N'‐dicyclohexylcarbodiimide (DCCD; an energy transfer inhibitor), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), antimycin A and potassium cyanide (electron transport inhibitors) have been extensively used to understand different biological processes in various organisms. In blue-green algae, effects of DNP on respiratory oxygen uptake in Synechococcus sp
and *Phormidium luridum* (Biggins, 1969), DCCD on nitrate uptake in *M. muscorum* (Rai et al., 1981), DCMU on photosynthesis in *Anabaena flos-aquae* (Stewart and Pearsen, 1970) and potassium cyanide on photophobic responses in *Phormidium uncinatum* (Hader, 1976) were studied.

Concerning the blue-green algal spore germination, most of the studies were done with DCMU, a specific inhibitor of photosystem II, to understand the role of photosynthesis in spore germination in *Anabaena cylindrica* (Yamamoto, 1976), *Anabaena variabilis* (Braune, 1979) and *M. muscorum* 7524 (Chauvat et al., 1982). In the present study, experiments were conducted to assess the influence of DNP (an uncoupler of oxidative phosphorylation), DCCD (an energy transfer inhibitor in photophosphorylation), DCMU (an inhibitor of electron transport in photosystem II), antimycin A and KCN (electron transport inhibitors of respiratory chain) on spore germination in *Modularia spumigena*. One of the aims of this investigation was conducted to ascertain the source of ATP generation which contributes to the energy demands of germinating spores.

In this study, spores obtained from the cultures grown in complete medium were employed. Experiments were conducted by supplementing complete HG medium with uncoupler/inhibitors one at a time. Investigations conducted without
the addition of these chemicals in medium served as controls. Methods of study are provided in the section dealing with material and methods of investigations (Method IIB:e v-ix, IV and V).

Results (Ref. Figs. 57 to 61)

DNP: In control as well as in the presence of DNP, lag in germination of spores was found to be similar (Fig. 57A). Inclusion of DNP in medium slightly lowered the percentage of germination as compared to control (Fig. 57A:a) and the percent inhibition of germination was found to be in proportion to the concentrations of DNP in medium (Fig. 57B).

DCCD: A lag in germination was similar both in control as well as in DCCD added treatments (Fig. 58A). Lower concentrations of DCCD (i.e., from 0.025 to 0.1 μM) stimulated the percentage of germination while higher concentration (0.5 μM) slightly inhibited it (Fig. 58A:a). About 18% increase in germination was observed at 0.025 μM concentration while 7% inhibition occurred at 0.05 μM concentration (Fig. 58B).

DCMU: In comparison to germination in control, inclusion of DCMU in medium resulted in the extension of lag of germination from 48 to 96 h (Fig. 59A). Further, it was also
found that the addition of different amounts of DCMU in medium brought down the percentage of germination from about 74 (control) to 54 depending on the concentration of the chemical (Fig. 59A:a). Percent decrease in germination was in proportion to the concentration of DCMU in medium (Fig. 59B).

Antimycin A: Addition of antimycin A, up to concentration of 10 nM, did not affect lag period of germination and it was found to be similar to control (Fig. 60A). On the contrary, in higher concentrations of antimycin A (20 and 40 nM), spores exhibited a lag in germination up to 120 h. It was found that at 168 h, the percentage germination was similar both in control and in medium containing 10 nM antimycin A. In higher concentrations (20 and 40 nM), the percentage germination decreased drastically (Fig. 60A:a) and resulted in 80% inhibition of germination (Fig. 60B).

Potassium cyanide: Inclusion of KCN in medium slightly enhanced the percentage of germination up to a period of 48 h, as compared to control (Fig. 61A). However, in comparison to control, at 168 h, germination percentage was lower in medium where potassium cyanide was added (Fig. 61A:a) and the percent inhibition was found to be in proportion to the amount of the inhibitor added (Fig. 61B).
Sequence of germination: Sequence of germination was similar in controls as well as in the treatments where DCCD, antimycin A or potassium cyanide were added. In these conditions, it was found that spores located near the mid-point of filament between heterocysts germinated first and germination progressed gradually towards heterocysts. On the contrary, in the presence of DNP and DCMU, spores adjacent to heterocysts germinated first and germination gradually progressed towards the mid-point of filament between heterocysts.

Discussion

In the present investigation, it was observed that inclusion of different amounts of DCMU in medium lowered the percentage of germination from about 74 (control) to 54 (i.e., 16 to 28% inhibition; depending on the concentration of the chemical used) but did not annul germination completely. The ability of majority of spores to germinate even in the presence of 20 μM DCMU suggests that ATP produced through non cyclic photophosphorylation and fresh production of photosynthates may not be necessary for germination as it was reported that DCMU completely abolishes photosynthesis by inhibiting photosynthetic electron flow (Izawa and Good, 1972). Braune (1979) working with *Anabaena variabilis*, reported the germination of spores in which photosystem II was completely inhibited by DCMU. Therefore, Braune (1979)
concluded that ATP generated by electron transport in photosystem II and fresh formation of photosynthates are not necessary for germination of spores. In addition, Chauvat et al. (1982) found that energy requirement for spore germination in Rostoc 7524 is also not efficiently fulfilled even by cyclic photophosphorylation on photosystem I.

In the present investigation, it was found that low concentrations of DCCD (up to 0.1 µM) stimulated germination while the concentration above 0.25 µM resulted in a slight inhibition of germination. As the inclusion of DCCD in medium did not largely inhibit germination, it may be suggested that the energy required for germination processes is not derived from photophosphorylation since DCCD, at the concentrations used in the present study, completely prevents ATP production through photophosphorylation by inhibiting transfer of free energy from electron transport to ATPase system in thylakoids (McCarty, 1980).

In Nodularia spumigena, it was found that even in the presence of 3 mM DNP (the highest concentration used in the present study) the decrease in germination was only by about 9% as compared to control (Fig. 57B). This indicates that the concentration of DNP used in the present study showed a mild effect on germination. Germination of majority of spores even in the presence of 3 mM
DNP indicates that the energy (ATP) required for germination processes in spores is probably not derived, at least to a large extent, from oxidative phosphorylation, since the presence of DNP (1 to 3 mM) stops the generation of ATP through oxidative phosphorylation process by specifically uncoupling the ATPase system from phosphorylating electron flow (Neumann and Jagendorf, 1964; see Lehninger, 1976).

The results obtained by using DCCD and DNP indicated that oxidative- and photophosphorylations may not be ATP providing sources for germination processes in Modularia spumigena. It is probable that ATP needed to meet energy requirements of germinating spores may be present as endogenous ATP pool and is sufficient to promote germination. Alternatively ATP required for germination processes may also come from substrate level phosphorylation.

Antimycin A and potassium cyanide are specific inhibitors of electron transport in respiratory chain between cyt b and cyt c₁ (site of inhibition by antimycin A) and between cyt a₃ and oxygen (site of inhibition by potassium cyanide). When respiratory electron transport is blocked it results in non oxidation of respiratory substrates as well as inhibition of ATP generation by oxidative phosphorylation. In the present investigation, germination in Modularia spumigena was inhibited by about 80% in the presence of
antimycin A (40 nM) and by about 40% in the presence of potassium cyanide (1 mM). The inhibition of ATP production as a consequence of blockage of electron transport in respiratory chain by antimycin A and potassium cyanide may not be a reason for the reduction of germination since studies with DNP indicated that ATP generated through oxidative phosphorylation is not the source of energy for the promotion of germination. Under these circumstances, the only probable reason for the inhibition of germination in the presence of antimycin A or potassium cyanide is non oxidation of some substrate(s) which may have to be oxidized for promoting spore germination in Modularia spumigena. Further work is necessary in order to ascertain the nature of the substrate(s) which may have to be oxidized to bring about spore germination. It may be worthwhile to mention here that spores of Modularia spumigena accumulate ascorbic acid during their development (see page 118).

Germination of about 60% of spores in the presence of potassium cyanide suggest the existence of cyanide insensitive respiratory pathway in these cells. In fact, existence of cyanide insensitive alternative oxidase system in respiratory chain has been reported in many bacteria and higher plants (see Thomas et al., 1973; Nicholls, 1982).

In completely sporulated filaments, the spores
formed adjacent to heterocysts germinated efficiently in the presence of DNP and DCMU while the spores produced away from heterocysts readily germinated in the presence of DCCD, antimycin A and potassium cyanide. These findings seem to indicate that the processes of germination in the spores produced adjacent to heterocysts are totally independent of ATP generated by oxidative phosphorylation and fresh formation of photosynthates whereas germination processes in the spores which are formed away from heterocysts are completely independent of ATP generated by photophosphorylation and necessity of oxidation of unknown substrate(s) by respiratory electron transport system.