

**PHYSIOLOGICAL STUDIES ON SPORES (AKINETES) OF THE BLUE-GREEN
ALGA *NODULARIA SPUMIGENA***

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**ABSTRACT OF THE THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT
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differentiation could be observed, various biochemical changes take place in the vegetative cells; these include increase in polyphosphate and polyphosphate contents, ATPase, leucine aminotransferase, succinate and TCA cycle activities, increase in arginine and lysophospholipids. However, activities of 2-oxoglutarate decarboxylase and glutamate decarboxylase are restored in vegetative cells after the vegetative changes.

Spore forming blue-green alga **Nodularia spumigena** collected from rice fields in Assam, India, was grown in cultures and used in the present study. Three different aspects viz., cytochemistry of spore differentiation, sporulation and spore germination were studied.

In **Nodularia spumigena**, spore formation commences in the centre of interheterocyst interval of filament and it gradually progresses towards heterocysts.

Investigations were conducted by employing cytochemical methods to understand spore differentiation and pattern formation in **Nodularia spumigena**. Cytochemical analysis revealed that the cells in filaments of **Nodularia spumigena** exhibited many biochemical changes not only during spore development but also before initiation of differentiation. 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, 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 activity was retained.

Polysaccharide and polyphosphate contents, and ATPase, L-aspartate:2-oxoglutarate aminotransferase and TTC reducing activities showed gradients in filament, with most polysaccharides/polyphosphates/ATPase activity/L-aspartate:2-oxoglutarate aminotransferase activity/TTC reducing activity present in the vegetative cells next to heterocyst and their concentration progressively decreasing towards the center of interheterocyst interval. Since decrease in the activities of ATPase and L-aspartate:2-

oxoglutarate aminotransferase leads to lowering in the production of ATP and aspartic acid, respectively, it seems that reduction in the amounts of ATP and aspartic acid to certain threshold levels are essential for promotion of spore initiation processes in vegetative cells. Nevertheless, decrease in polysaccharides, polyphosphates and TTC reducing activity in vegetative cells also seem to be equally responsible for initiation of spore differentiation.

Initiation of spore differentiation took place subsequent to reduction in polysaccharides/polyphosphates/ATPase activity/TTC reducing (electron transport) activity/L-aspartate:2-oxoglutarate aminotransferase activity in the centre of interheterocyst interval as a result of gradients established by heterocysts. This implies that heterocysts influence/regulate the formation of gradients which inturn control regularity in spacing of spore differentiation thereby establishing a pattern in spore formation, i.e, spore formation commencing at mid-point of filament between heterocysts and gradually progressing towards heterocysts.

Accumulation of arginine and tryptophan in vegetative cells preceeding/during spore initiation stage suggest the involvement of these amino acids in metabolic pathways leading to the initiation of spore differentiation.

During spore development L-aspartate:2-oxoglutarate aminotransferase activity was restored. The accumulation of aspartic acid (as indicated by amino acid analysis) in matured spores might have resulted from the restoration of activity of this enzyme in the cells during their development into spores.

Development of detectable activities of alkaline and acid phosphatases including glucose-6-phosphatase in developing spores suggest that these enzymes may be performing a role of removing phosphate from differentiating spores.

Effects of various factors like quality of light, trace elements, nitrate, phosphate, pH, amino acids, growth regulators and metabolic inhibitors on sporulation and spore germination were investigated.

Effect of different spectral bands of light on sporulation and spore germination was studied. Both white and red lights promoted sporulation and germination equally well. Blue and green lights supported sporulation but drastically inhibited germination. Further blue light was more stimulatory for sporulation than green light while the effects of these lights on germination, were similar. These findings evidence the varied effects of blue and green

lights on sporulation and spore germination.

Investigations were performed to assess the effects of trace elements on sporulation and germination. In the absence of trace elements both sporulation and germination were affected. Sporulation was maximally inhibited when copper was excluded from the medium and it was followed by zinc, manganese and cobalt. On the other hand, germination was maximally inhibited when cobalt was removed from the medium and it was followed by copper, manganese and zinc. These findings indicate relative importance of these micronutrients in sporulation and germination processes.

Effect of nitrate on sporulation and germination was studied. Exclusion of nitrate from the medium affected germination much more than sporulation. Failure of some spores to germinate inspite of having cyanophycin material (nitrogen reserve) in them indicates that the nitrogen reserves in these spores either not fully utilized or insufficient for the completion of germination processes.

Effect of phosphate on sporulation and germination was investigated. Phosphate starvation hastened the process of sporulation and time duration for maximum sporulation was reduced to nearly half. On the other hand, removal of phosphate from the medium drastically affected germination.

From these findings it is clear that the effect of phosphorus is variable with regard to sporulation and spore germination.

Effect of pH on sporulation and spore germination in the presence or absence of nitrate and phosphate was investigated. In the medium lacking nitrate, pH 6 to 7 and 10 to 11 resulted in increased sporulation compared to control. But such a stimulation of sporulation was not observed in complete medium as well as in the medium lacking phosphate. These findings indicate that the pH optima for sporulation varies depending on the presence or absence of nitrate or phosphate in the medium. In case of germination, optimum pH conditions for maximum germination shifted down from 9-11 in complete medium to 9 in the medium lacking nitrate and 8 in the medium devoid of phosphate. These findings suggest that depending on the type of nutrient deficiency, different germination system, existing in the spores, may be activated at different pH optima.

Studies were conducted to ascertain the effects of exogenously supplied amino acids, viz., L-arginine, L-aspartic acid, L-isoleucine, L-leucine, L-phenylalanine and L-tryptophan on sporulation and spore germination in the presence or absence of nitrate in medium. In the medium devoid of nitrate, exogenous supply of L-arginine and L-tryptophan enhanced sporulation up to a considerable level.

However, only L-arginine allowed normal sporulation in the medium containing nitrate. In case of germination, addition of amino acids in lower concentrations in the medium lacking nitrate was found to be stimulatory while similar concentrations of amino acids in the medium containing nitrate lowered germination. Reason for inhibition of sporulation and germination by L-aspartic acid, L-isoleucine, L-leucine and L-phenylalanine in the presence of nitrate is not known. Stimulation of sporulation in the presence of L-arginine and L-tryptophan suggest that they play an important role in spore differentiation.

Effect of growth regulators, viz., 2,4-dichlorophenoxy acetic acid (2,4-D), indole acetic acid (IAA), Kinetin and gibberellic acid (GA_3) on sporulation and spore germination was studied. Excepting in low concentrations of 2,4-D, sporulation was largely inhibited by other growth regulators. In case of germination, 2,4-D and IAA stimulated the process only when added at lower concentrations while kinetin and GA_3 stimulated germination when supplied both at lower and higher concentrations.

Effect of 2,4-dinitrophenol (DNP; an uncoupler of oxidative phosphorylation), N,N'-dicyclohexylcarbodiimide (DCCD; energy transfer inhibitor in photophosphorylation), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU; electron

transport inhibitor of photosynthesis), antimycin A and potassium cyanide (electron transport inhibitors in respiratory chain) were studied on spore germination while effect of DNP, DCCD and DCMU was studied on sporulation.

Addition of DNP resulted in moderate inhibition of sporulation but its effect on germination was negligible. Moderate inhibition of sporulation in the presence of DNP suggests that the energy (ATP) needed for sporulation processes is probably derived, at least partly, from oxidative phosphorylation. On the other hand, energy required for germination processes seem to be independent of ATP generated by oxidative phosphorylation.

DCCD exhibited a minimal inhibition of both sporulation and germination. This implies that ATP needed for these processes is not fulfilled by photophosphorylation.

In the presence of DCMU, sporulation was drastically reduced but not germination. This finding suggests that fresh formation of photosynthates is necessary for promoting spore formation but not germination.

Antimycin A and KCN inhibited germination of spores. However, antimycin A affected germination much more than KCN. Since the inhibition of ATP, generated as a result of respiratory electron flow (oxidative phosphorylation),

not
is found to be a reason for decrease in germination, then
the inhibition of germination in the presence of antimycin
A or KCN suggests that oxidation of some substrate which
is brought about by respiratory reactions is necessary
to promote germination. Further, germination of about 60%
of spores in the presence of KCN indicates existence of
cyanide insensitive respiration in spores of *Nodularia
spumigena*.

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