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Spore Germination in *Cylindrospermum* sp.: Influence of Gases and Growth Conditions

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ABSTRACT. Influence of different gases on spore germination of *Cylindrospermum* sp. revealed that acetylene and ethylene suppressed the germination which was more pronounced at their higher concentrations. Germination was completely inhibited under the atmosphere of hydrogen and argon even when supplied with 1 % CO_2. Carbon dioxide, both in air and in N_2, stimulated the process. However, higher concentrations of CO_2 inhibited the germination in a concentration-dependent manner, possibly due to a decrease in pH. Germination was completely arrested in the absence of light and nutrients. The energetic requirement for germination was not efficiently fulfilled by photosystem I (PS II was blocked by 3-(3,4-dichlorophenyl)-1,1-dimethylurea) or under conditions of anoxygenic photosynthesis (medium supplemented with sodium sulfide).

Cyanobacteria (blue-green algae) belonging to orders *Nostocales* and *Stigonematales* (section IV and V; Rippka *et al.* 1979) differentiate spores (akinetes) under unfavourable growth conditions serving as means of perennation (Nicholas and Carr 1978). Although spore germination in cyanobacteria was observed as early as 1757 by Reumer, little work has been done on the germination process as compared to bacterial or fungal spores. It is likely that spores in cyanobacteria may differ structurally and in their metabolic potentialities from vegetative cells. They usually contain a high amount of phycocyanin and glycogen (Sutherland *et al.* 1979) serving as nitrogen and carbon sources, respectively, during the germination (Sutherland *et al.* 1985). Cyanobacterial spores have decreased pigment contents, CO_2-fixing capacity and photosynthetic activity (Nicholas and Adams 1982).

The effect of different gases, such as acetylene and ethylene, proved to be inhibitory for sporulation in *Cylindrospermum licheniforme* and hydrogen stimulated the process (Hirosawa and Wolk 1979). The first such study on the effect of

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different gases on spore germination of *Nostoc* PCC 7524 showed that changes in gas phases from N_2:CO_2 to air:CO_2 altered the inhibitory action of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and spore germinated, although at a low frequency. In the present communication we describe the influence of different gases and growth conditions on spore germination of *Cylindrospermum* sp.

MATERIALS AND METHODS

The *Cylindrospermum* species used was isolated from a local rice field. The cyanobacterium was cultivated in modified Chu-10 medium (Safferman and Morris 1964) with A-6 trace elements (Allen and Arnon 1955) under photoautotrophic growth conditions at 25 ± 1 °C in a culture room (illuminated with cool day-light fluorescent tubes; intensity 2.5 klx; 14/10 h light–dark cycle). The spores formed during early stationary growth phase were harvested by centrifugation, washed twice with sterilized double-distilled water and were used for germination studies. To obtain synchrony in germination the spores were pre-treated in the dark for 4 d before harvesting. All the experiments were conducted in Venoject vacuum blood collection tubes (*Terumo Medical Corporation*, USA) fitted with an air-tight rubber stopper under standard growth conditions as described for cultivation of cyanobacteria. Some gas phases (H_2, N_2 and argon) were obtained by the water displacement method, repeated twice to ensure the desired gas phase. Other (CO_2, ethylene and acetylene) were injected into the tubes after removing the equivalent volume of air/gas. After the addition of filter-sterilized sodium sulfide, the pH of the medium was adjusted to 7.8 (pH of the basal medium). Spores (5×10^6/mL) were inoculated in each tube. Each treatment was performed in triplicate and the mean values are presented herein.

RESULTS

Figure 1 shows the time course of spore germination. When the spore suspension was inoculated in fresh mineral medium and incubated under growth conditions, the first doublets (spore containing two cells) appeared after a lag of 1 d. These doublets were considered as the first sign of spore germination. The frequency of such doublets was 25 % and increased with the incubation period. After 2 d of incubation both 2-celled and 4-celled stages were observed. Synchrony of 4-celled stage was observed after 3 d, liberated after rupturing the spore coat after 3–4 d of incubation. At this stage, 20 % of germling population differentiated heterocysts at one of the apical cell.

Acetylene and ethylene suppressed spore germination which was more pronounced at higher concentrations. Ethylene was more lethal than acetylene and complete inhibition of spore germination was observed at 1.25 and 2 % gas volume of ethylene and acetylene in air, respectively. Spore germination was not observed under anaerobic growth conditions created by H_2 or argon even when supplemented

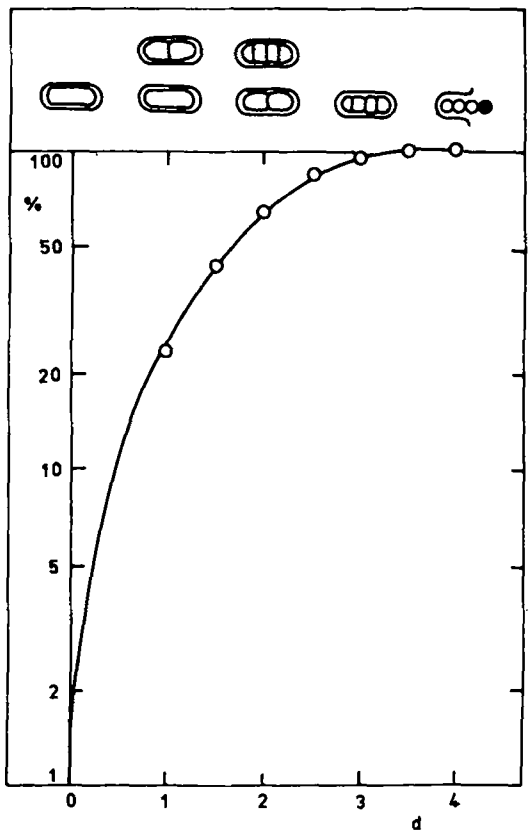

FIG. 1. Kinetics of spore germination (%) of *Cylindrospermum* sp. under standard conditions. Frequency of germination includes doublets, 4-celled stage, and liberated germlings.

with 1 % CO_2. Carbon dioxide both in air and nitrogen stimulated spore germination. However, increasing concentrations (5 – 10 %) of CO_2 suppress the process (Table I).

Spore germination was completely arrested in the photoheterotrophic growth conditions (supplemented with glucose or sucrose, 1 %, *W/V*) or in the absence of mineral nutrients under photoautotrophic mode of nutrition. Spore germination was observed at a low frequency in the presence of sodium sulfide (3 mmol/L) or DCMU (15 μmol/L) (Table II).

DISCUSSION

Dinitrogen fixation, like in bacteria, coupled with CO_2 fixation similar to higher plants, are the unique characteristics of cyanobacteria. They evolve and metabolize H_2, the former being maximum under anaerobic condition created by argon supplementation (Spiller *et al.* 1978). Nitrogenase (EC 1.18.6.1) reduces

TABLE I. Influence of different gases on spore germination of *Cylindrospermum* sp.

Gases, %							Spore germination (%) after	
Air	C_2H_2	C_2H_4	H_2	Ar	N_2	CO_2	3 d	4 d
99.5	0.5						64	98
99	1						28	46
98.5	1.5						3.6	12
98	2						0	0
99.75		0.25					58	84
99.5		0.5					25	54
99		1					2	9.6
98.75		1.25					0	0
			100				0	0
			99			1	0	0
				100			0	0
				99		1	0	0
					99	1	86	100
99						1	82	100
97.5						2.5	68	97
95						5	57	88
90						10	46	72
100							65	97

Statistical analysis of variance (ANOVA) has shown that germination significantly varied between the concentrations of a particular gas and also between different gases
(Concentration $F_{3,22} = 230$, $P < 0.001$; Gas treatments $F_{2\ 22} = 581$, $P < 0.001$.

acetylene to ethylene and acetylene behaves as a competitive inhibitor for N_2 fixation (Schollhorn and Burris 1967). However, the influence of these gases on sporulation and spore germination of cyanobacteria is not well understood (Sutherland *et al.* 1979; Hirosawa and Wolk 1979; Chauvat *et al.* 1982). Hydrogen gas stimulated the sporulation in *C. licheniforme* which may be confined to the

TABLE II. Effect of different growth conditions on spore germination of *Cylindrospermum* sp.

Growth conditions	Spore germination (%) after 4 d ± S.E.
Light (control)	96.8 ± 0.806
DCMU (15 μmol/L)	20.6 ± 0.985
Sodium sulfide (3 mmol/L)	21.0 ± 1.180
Dark	0
Dark (glucose 1 %)	0
H_2O (mineral medium)	0

heterocyst-mediated effect as the hydrogenase (EC 1.18.99.1) activity was shown to be located solely in the heterocyst (Hirosawa and Wolk 1979). Such a possibility cannot be ruled out for spore germination as the spores are structurally and metabolically different from the heterocysts. Higher concentrations of H_2 used in the present investigation, as required for hydrogenase assay (Peterson and Burris 1978), inhibited the spore germination. It is difficult to explain the underlying reasons, however; failure of spore germination could be due to inhibition of some critical step(s) in spore germination, possibly unbalancing energy pathway. Although 2-chloroethanol (ethylene chlorohydrin) has been used for breaking bud dormancy and although ethylene contributes to radial expansion of tuber it did not aid germination but rather it inhibited the process. A continuous supply of carbon in the form of CO_2 is required for sporulation in *Nostoc* PCC 7524 (Sutherland *et al.* 1979) and for spore germination in the present investigation where its absence inhibited germination. The failure of spores to germinate under H_2 : CO_2 or argon: : CO_2 atmosphere might be due to the anaerobic conditions known to inhibit spore germination in *Anabaena cylindrica* (Yamamoto 1976). Although akinete germination was reported in *Nostoc* PCC 7524 under argon: CO_2 in the absence of nitrogen sources (Sutherland *et al.* 1985), CO_2 in excess reduced the germination which might be attributed to a decrease in pH. Both acid or alkaline pH values are known to inhibit the germination of spores of *A. cylindrica* (Yamamoto 1976) and *Anabaena vaginicola* (Rai and Pandey 1981).

Light as an environmental factor may operate to regulate sporulation as well as spore germination of several cyanobacteria (Nicholas and Carr 1978; Nicholas and Adams 1982; Pandey and Kashyap 1987). It is also possible that the energy requirement for germination is solely met from photosynthesis as spore germination was completely arrested in the dark even in the presence of an organic carbon source

in *Nostoc* PCC 7524 and in the present investigation. DCMU, an inhibitor of PS II and thus of CO_2 fixation blocked germination either completely (Yamamoto 1976) or only slightly (Braune 1979). However, change in the gas phase from N_2 : CO_2 to air : CO_2 relieved the effect of DCMU and spore germination was observed although with a low frequency (Chauvat *et al.* 1982). A low frequency of spore germination in the present investigation indicates that PS I activity may alone support the energy requirement for germination although partially. It is also possible that DCMU may not be fully effective in PS II inhibition since it was completely inhibitory only under anaerobic conditions (Chauvat *et al.* 1982). When conditions for oxygenic photosynthesis were shifted to anoxygenic by using sodium sulfide it did not support rapid germination. It seems that oxygen tension created by reducing conditions may not initiate spore germination. The situation is similar to the observation of Chauvat *et al.* (1982) who showed that respiratory activity appeared to stimulate germination of *Nostoc* PCC 7524 spores when PS I was operative in the absence of PS II and when oxygen was present.

In conclusion, spores required light energy, operation of both photosystems and aerobic, CO_2-rich, atmosphere besides the mineral nutrients for germination.

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REFERENCES

ALLEN M.B., ARNON D.I.: Studies on nitrogen fixing blue-green algae. I. Growth and nitrogen fixation by *Anabaena cylindrica* LEMM. *Plant Physiol.* **30**, 366–372 (1955).

BRAUNE W.: *C-phycocyanin* – the main photoreceptor in the germination process of *Anabaena akinetes*. *Arch.Microbiol.* **122**, 289–295 (1979).

CHAUVAT F., CORRE B., HERDMAN M., JOSET-ESPARDELLIER F.: Energetic and metabolic requirements for germination of akinetes of the cyanobacterium *Nostoc* PCC 7524. *Arch.Microbiol.* **133**, 44–49 (1982).

HIROSAWA T., WOLK C.P.: Factors controlling the formation of akinetes adjacent to heterocysts in the cyanobacterium *Cylindrospermum licheniforme* KUTZ. *J.Gen.Microbiol.* **114**, 423–432 (1979).

NICHOLAS J.M., ADAMS D.G.: Akinetes, p. 387–412 in N.G. Carr, B.A. Whitton (Eds.): *The Biology of Cyanobacteria.* Blackwell Scientific Publications, Oxford 1982.

NICHOLAS J. M., CARR N.G.: Akinetes of cyanobacteria, p. 335–343 in G. Chambliss, J.C. Vary (Eds.) *Spores VII.* American Society of Microbiology, Washington 1978.

PANDEY K.D., KASHYAP A.K.: Factors effecting formation of spores (akinetes) in cyanobacterium *Anabaena doliolum* (AdS strain). *J.Plant Physiol.* **127**, 123–134 (1987).

PETERSON R.B., BURRIS R.H.: Hydrogen metabolism in isolated heterocysts of *Anabaena* 7120. *Arch.Microbiol.* **116**, 125–132 (1978).

RAI A.K., PANDEY G.P.: Influence of environmental stress on the germination of *Anabaena vaginicola* akinetes. *Ann.Bot.*. **48**, 361–370 (1981).

RIPPKA R., DERUELLES J., WATERBURY J.B., HERDMAN M., STANIER R.Y.: Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J.Gen.Microbiol.* **111**, 1–61 (1979).

SAFFERMAN R.S., MORRIS M.E.: Growth characteristics of blue-green algal virus LPP-1. *J.Bacteriol.* **88**, 771–775 (1964).

SCHOLLHORN R., BURRIS R.H.: Acetylene as a competitive inhibitor for N_2-fixation. *Proc.Nat.Acad. Sci.USA* **58**, 213–216 (1967).

SPILLER H., ERNST A., KERFIN W., BOGER P.: Increase and stabilization of photoproduction of hydrogen in *Nostoc muscorum* by photosynthetic electron transport. *Z.Naturf.* **33**, 541–547 (1978).

SUTHERLAND J.M., HERDMAN M., STEWART W.D.P.: Akinetes of cyanobacterium *Nostoc* PCC 7524: macromolecular composition, structure and control of differentiation. *J.Gen.Microbiol.* **115**, 273–278 (1979).

SUTHERLAND J.M., STEWART W.D.P., HERDMAN M.: Akinetes of the cyanobacterium *Nostoc* PCC 7524: morphological changes during synchronous germination. *Arch.Microbiol.* **142**, 269–274 (1985).

YAMOTO Y.: Effect of some physical and chemical factors on the germination of akinetes of *Anabaena cylinidrica*. *J.Gen.Appl.Microbiol.* **22**, 311–323 (1976).