Effects of temperature pre-treatment, desiccation and aging on the viability of spores of halophilic blue-green algae

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Abstract

The effects of temperature, desiccation and aging on the viability of spores of Sambhar salt lake blue-green algae, *Anabaena fertilissima* and *Anabaenopsis arnoldii*, were studied. Spores of *A. arnoldii* were found to be more susceptible to temperature variation, desiccation and storage than spores of *A. fertilissima*. Pre-treatment of spores with higher temperatures, 37° and 47° C, stimulated germination in *A. fertilissima*.

In a sporulated filament, spores which developed first were generally bigger and more resistant to adverse environmental conditions than spores formed later. The differential loss of viability in spores of a filament may be due to certain intrinsic differences in the physiological/biochemical properties of the spores.

Introduction

Blue-green algae show a wide range of adaptability. During adverse growth conditions some heterocystous blue-green algae produce spores (akinetes) capable of withstanding such conditions better than vegetative cells. Though information regarding the ability of spores of freshwater blue-green algae to survive desiccation and temperature is available (Yamamoto, 1975, 1976; Sutherland *et al.*, 1979), nothing is known about spores of salt water blue-green algae. Further, no comparative studies have been made on factors that affect germinability of spores of blue-green algae occupying the same habitat in nature. Such studies may provide information about the strategies adopted by different algae for their establishment, survival and growth. In this paper, effects of temperature pre-treatment, desiccation and aging on viability of spores of *Anabaena fertilissima* and *Anabaenopsis arnoldii* are reported.

Materials and methods

Clonal cultures of *A. fertilissima* and *A. arnoldii* (both isolated from Sambhar salt lake, Rajasthan) were grown in Sambhar salt medium (SSM) at pH 7.0 and 8.5, respectively (Reddy *et al.*, 1975). The cultures were maintained at a temperature of 28 ± 1 °C and light intensity of 2 400 lx supplied continuously by 40 W daylight fluorescent lamps. Under these conditions all the vegetative cells of the filaments of both blue-green algae developed into spores (in catenate series) within 15–20 days. Fully matured spores were harvested, washed, homogenized and diluted ($\leq 10^4$ spores ml^{-1}) in the medium and used in the investigations. Germination studies were done in petriplates containing agarized (1.5%) SSM.

The spores collected from one month old cultures were used for studying the effects of temperature and desiccation on germination.

Temperature pre-treatment

Equal amounts of spore suspension were taken in

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four sets of tubes and incubated in the dark at 0°, 7°, 37° and 47° C. At every 3, 6, 12, 24 and 48 h intervals, one tube from each set was taken out and 1 ml of spore suspension was plated on agarized medium. The spores which were directly plated without temperature pre-treatment but stored at 28 ± 1° C served as control.

Desiccation

Equal amounts of spore suspension were placed on grease-free microslides, air-dried and kept in desiccators containing $CaCl_2$ or H_2SO_4. The desiccators were kept in the dark at a temperature of 28 ± 1° C. At intervals of 24, 48, 72, 96, 120, 144, 192 and 240 h, the slides were taken out, the spore material was scraped and dispersed in a small volume of sterile SSM and spread on nutrient agar plates. The undesiccated spores plated simultaneously served as control.

Storage

The spores harvested from 1–4 months old liquid cultures kept at 28 ± 1° C and 2 400 lx were employed for investigating the effect of storage/aging on germination.

The spores plated on agarized medium were incubated at 28 ± 1 ° C and 2 400 lx. The number of germinated and ungerminated spores present in seventy five randomly selected microscopic fields from triplicate plates (for each treatment) was counted and percentage germination was calculated at 24 h intervals.

Results and discussion

Effect of temperature pre-treatment

In the controls, a lag in germination was not observed in either alga. On the other hand, spores of *A. fertilissima* pre-treated at 0°, 7°, 37° and 47° C showed a lag in germination for a duration of 24 h (Fig. 1). In *A. arnoldii*, the spores pre-treated at 7° and 37° C exhibited a lag in germination for 24 h. This lag phase extended up to 48 h when spores were treated at 0° and 47° C.

In *A. fertilissima*, spores pre-treated at 0° and 7° C attained an almost stationary level of germination after about 120 h, while this was attained at 72 h when pre-treated with 47° C (Fig. 1A, B, E). However, spores pre-treated at 37° C and in control reached a stationary level of germination by 96 h (Fig. 1C, D). In *A. arnoldii*, an almost stationary level was attained by 96 h both in control as well as pre-treated spores at all temperatures (Fig. 1A–E).

In *A. arnoldii*, pre-treatment at 0°, 7°, 37° and 47° C, even for 3 h, drastically brought down germination from 62% (control) to 10%, 18%, 7% and 3%, respectively, while in *A. fertilissima*, there was only a slight reduction in germination. Pre-treatment of spores of *A. fertilissima* at 0° C lowered the percentage germination more than other temperature treatments. In both algae, increased duration of pre-treatment (3 to 48 h) at all temperatures brought about consistant decrease in germination. On the other hand, no germination was observed in spores of *A. arnoldii* pre-treated at 0° C for more than 12 h and in spores treated at 47° C beyond 24 h.

These results indicate that spores of *A. fertilissima* survive both low (0° and 7° C) and high (37° and 47° C) temperatures quite effectively while spores of *A. arnoldii* show a drastic reduction in germination when subjected to temperature treatments. This suggests that spores of *A. fertilissima* are more resistant to variations in temperature than spores of *A. arnoldii*. Such a differential response has also been reported in fresh water blue-green algae. Spores of *Cylindrospermum majus* survived temperatures up to 100° C (Glade, 1914) while in spores of *Anabaena cylindrica*, temperatures higher than 55° C resulted in complete loss of viability (Yamamoto, 1976).

In nature, *A. fertilissima* and *A. arnoldii* grow together in Sambhar salt lake were the temperature falls as low as 0° C in winter and rises to 50° C during summer. Under natural conditions, *A. arnoldii* starts sporulating by the end of winter or the beginning of spring while *A. fertilissima* starts sporulating in late summer. Though both algae reappear during the following rainy season, *A. arnoldii* forms a bloom, while *A. fertilissima* occurs sparsely. Spores collected during summer from the sediments of the lake, when tested for viability, showed about 50% germination in *A. arnoldii* and 97% in *A. fertilissima*. Thus it appears that in natural conditions about half the spores of *A. arnoldii* can withstand the high temperatures of summer.

However, spores formed in cultures did not show such a degree of thermoresistance. Löwenstein (1903) found that *Mastigocladus laminosus*, which grows in nature at 52° C, loses its capacity to survive at high temperatures on prolonged culturing at room temperature. He suggested that this was due to impairment of a particular biosynthetic system. In the present experiment the failure of spores obtained from laboratory cultures of *A. arnoldii* to germinate after temperature pre-treatment might be due to a similar reason. On the other hand, prolonged culturing of *A. fertilissima* did not result in any impairment in viability of spores. Its sparse occurrence in the lake may thus be due to its inability to compete with *A. arnoldii* and under natural conditions, its high germination may be a strategy

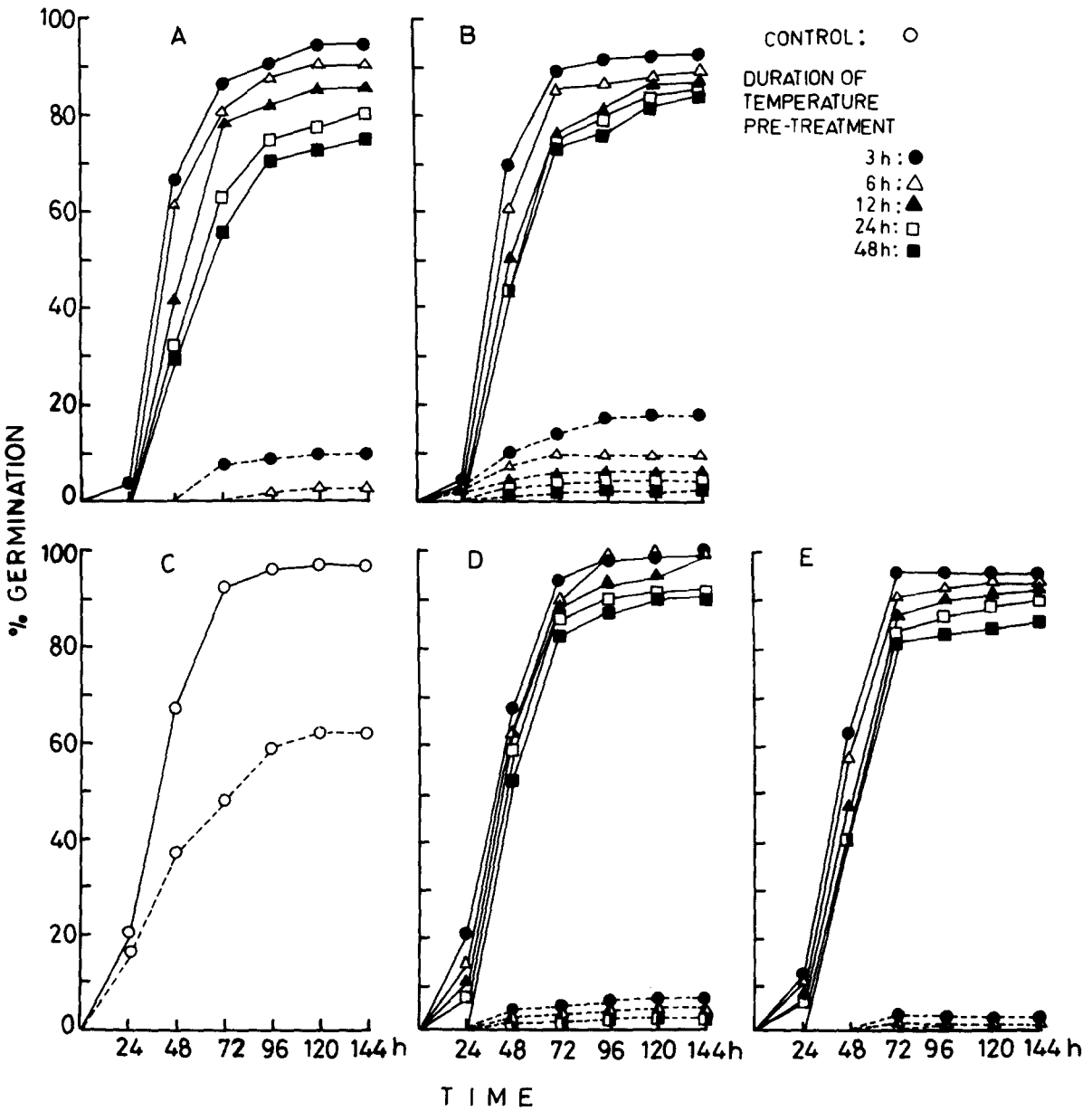

Fig. 1. Effect of temperature pre-treatment of spores of *Anabaena fertilissima* (—) and *Anabaenopsis arnoldii* (---) on germination; (A) 0° C, (B) 7° C, (C) 28 ± 1° C (Control), (D) 37° C and (E) 47° C.

Table 1. Mean of cells in germlings of spores of *Anabaena fertilissima* and *Anabaenopsis arnoldii* pre-treated with different temperatures.

Blue-green algae	Length of single cell	Number of replicates	Mean of cells (± S.E.) of germlings of spores pre-treated with different temperatures					Dipicolinic acid in spores
			Control	0°C	7°C	37°C	47°C	
A. fertilissima	7 μm	70	2.97 ± 0.05	1.99 ± 0.06	1.94 ± 0.07	5.9 ± 0.06	6.21 ± 0.05	absent
A. arnoldii	6.5 μm	66	3.05 ± 0.08	1.11 ± 0.04	2.02 ± 0.06	2.0 ± 0.06	1.14 ± 0.04	absent

ANOVA

Blue-green algae	Source of variation	D.F.	S.S.	M.S.S.	F
A. fertilissima	Between treatments	4	1236.61	309.15	1066.04**
	Error S.S.	345	100.79	0.29	
	Total S.S.	349	1337.4		
A. arnoldii	Between treatments	4	167.44	41.86	174.42**
	Error S.S.	325	76.86	0.24	
	Total S.S.	329	244.3		

to allow at least a small portion of germlings to establish themselves.

In both algae, pre-treatment of spores with various temperatures influenced the germling growth differently (Table 1, ANOVA). At 24 h of germination, the lengths of the germlings of spores of *A. fertilissima* pre-treated with higher temperatures, 37° C ($t = 32.13$, D.F. = 138, $p = .001$) and 47° C ($t = 31.95$, D.F. = 138, $p = .001$), are greater than those produced in the control (Table 1). The increase in length of the germlings may be due to stimulation of germination (fast germination) induced by pre-treatment with higher temperatures. The resistance/stimulation of germination of spores pre-treated at high temperatures suggests the presence of a mechanism which confers thermoresistance/thermostimulation to the spores. Fogg (1956) is of the opinion that blue-green algae survive at high temperatures by the stability of their enzymatic and protoplasmic structures.

Effect of desiccation

Almost identical results in germination were obtained when spores were desiccated either in presence of $CaCl_2$ or H_2SO_4.

In *A. fertilissima*, a lag of 48 h in germination was observed when the spores were desiccated for 72 h or more (Fig. 2A). However, spores of *A. arnoldii* exhibited a lag in germination for 24 h, only when desiccated for more than 196 h (Fig. 2B).

In *A. fertilissima*, a stationary level in germination was generally attained by 96 h in the control and by 120 h with desiccated spores. However, in *A. arnoldii*, spores in control attained an almost stationary level of germination at about 96 h while this was attained at 72 h when desiccated.

In both algae, the germination percentage gradually decreased as the desiccation period increased. The longer the desiccation, the lesser the rate of germination. The delay in germination or loss of viability of desiccated spores is evidently due to dehydration. Spores of *A. arnoldii* are more susceptible to desiccation than the spores of *A. fertilissima* (Fig. 2). Yamamoto (1975) also found that the spores of *A. cylindrica* gradually lost their ability to germinate when desiccated.

In both *A. fertilissima* and *A. arnoldii*, spores desiccated even for 24 h showed a sudden fall in germination; from 98% to 54% in *A. fertilissima* and from 62% to 28% in *A. arnoldii*. A sudden decrease in germination is evidently due to the presence of some spores which are highly susceptible to dehydration. However, the spores of *A. arnoldii* dried for 48–120 h showed almost the same percentage of germination (about 21%). Thus it is obvious that most of the spores which survived 24 h of de-

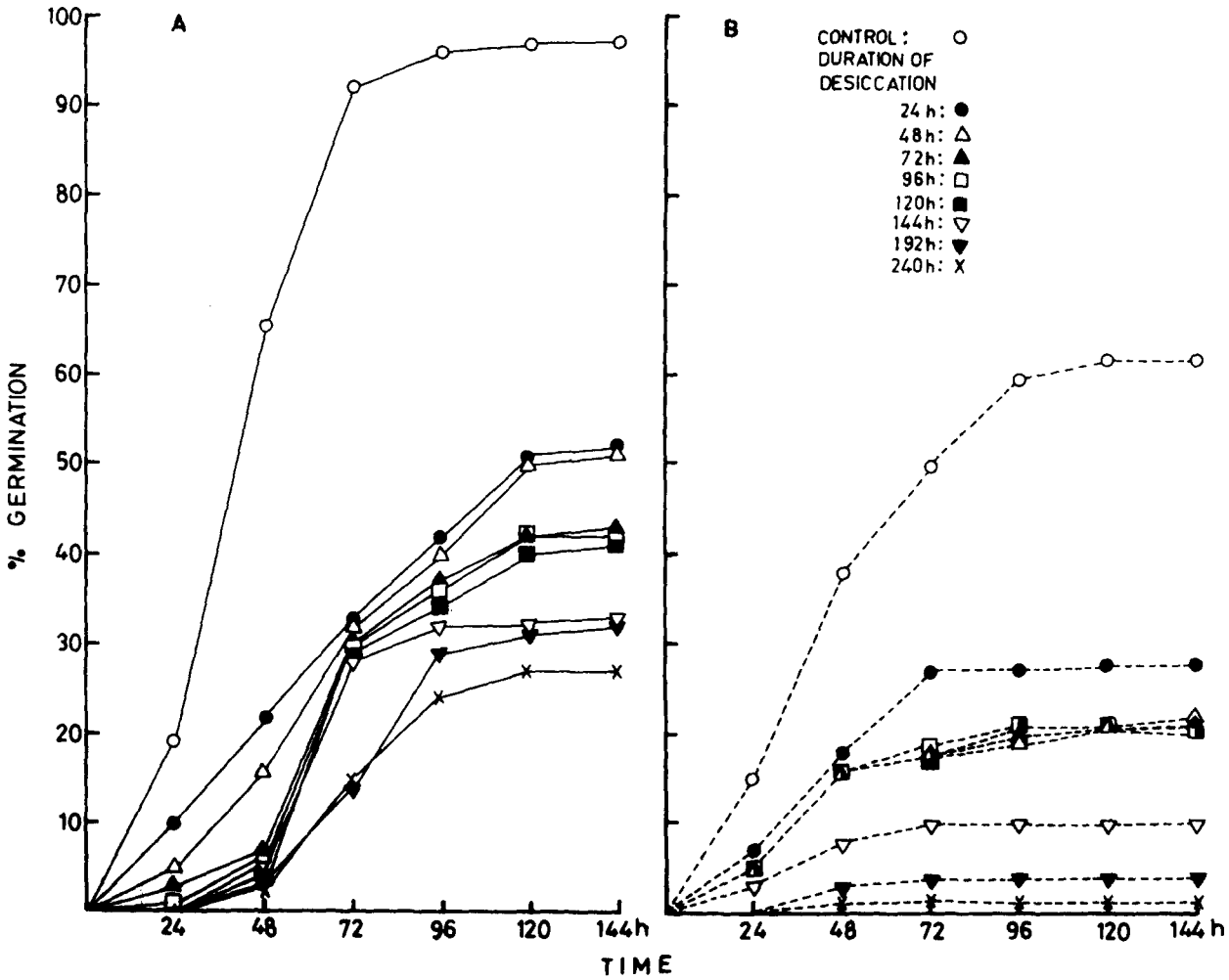

Fig. 2. Effect of desiccation of spores of (A) *Anabaena fertilissima* and (B) *Anabaenopsis arnoldii* on germination.

siccation can withstand drying up to 120 h without a further fall in germination. Soon after a threshold is reached (which is attained within about 120 h), the spores gradually lose their viability with further increase in desiccation period. These findings suggest that at least two types of spores (in relation to their capacity to withstand desiccation) exist in *A. arnoldii*. They can be classified as 1. spores sensitive to 24 h of desiccation and 2. spores resistant to 24 h of drying, but sensitive to desiccation beyond 120 h. On the other hand, in *A. fertilissima*, at 144 h of incubation, germination was about 52%, 42% and 33% in spores desiccated for 24–48 h, 72–120 h and 144–192 h, respectively. This suggests that there are at least three categories of spores in *A. fertilissima*: 1. Spores sensitive to 24–48 h of desiccation; 2. Spores resistant to 48 h of drying, but sensitive to desiccation of 72–120 h and 3. Spores resistant to 120 h of desiccation, but sensitive to drying beyond 144 h.

Effect of storage

In *A. fertilissima*, the germination percentage fell from 98 to 92 within 1.5 months of storage and thereafter remained almost constant. On the contrary, in *A. arnoldii*, spore germination gradually decreased from 62% to 37% within 3.5 months of storage (Fig. 3). Yamamoto (1975) reported that 75% of the spores of *A. cylindrica* lost viability when stored for 6 months at 27°C and 500 lx. In *A. fertilissima* and *A. arnoldii*, about 6% and 40% of spores lost viability, respectively, when stored for 4 months at 28 ± 1°C and 2 400 lx. It is evident

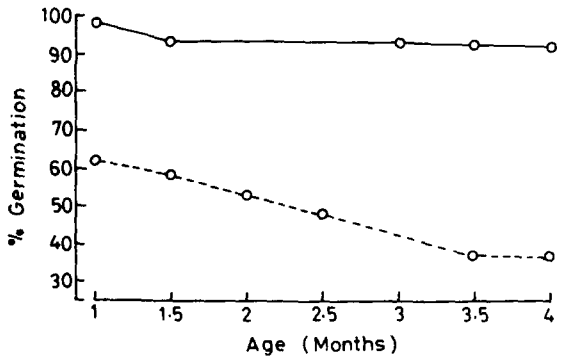

Fig. 3. Effect of storage on spore germination in *Anabaena fertilissima* (—) and *Anabaenopsis arnoldii* (---).

from the results that spores of *A. fertilissima* retain viability better than spores of *A. arnoldii* when stored for long duration.

That all members of a sample of spores from a given culture do not germinate simultaneously implies that individual spores vary in their susceptibility. In spores of *A. fertilissima* and *A. arnoldii*, heterogeneity is expressed in delayed germination. Moreover, populations of spores show heterogeneity to other parameters like resistance to temperature, desiccation and storage. Heterogeneity in the size of spores formed in a single filament was also observed. Both in *A. fertilissima* and *A. arnoldii*, the commencement of sporulation started about in the centre between two heterocysts and sporulation progressed towards them (Fig. 4A). After some time, the whole filament became completely sporogenous. Spores which developed first were bigger than the ones formed near to the heterocysts. In order to find out how spores with developmental differences respond to factors governing germination, intact sporulated filaments were subjected to temperature pre-treatment/desiccation/storage as described earlier and tested for germination. In both algae, it was found that spores adjacent to heterocysts lost viability faster than spores further away from the heterocysts. As the duration of temperature pre-treatment/desiccation/storage was increased, spores progressively lost viability in the reverse direction of sporulation (Fig. 4B–D). The findings evidence that in a chain of spores, those spores which are bigger and formed first have a better capacity to withstand adverse environmental conditions than the ones which are smaller and formed later. It is not known whether the resistance of spores to adverse conditions is due to morphological features or physiological properties. The differential loss of germinability may be due to intrinsic differences in the physiological/biochemical status of the spores, but morphological features (like thick spore envelope/-wall) in offering resistance to adverse conditions can also be important.

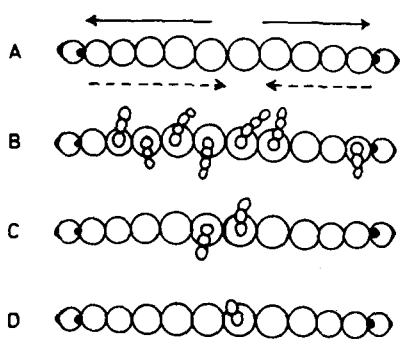

Fig. 4. (A) Diagrammatic representation showing the directions of sporulation (⟵) and progressive loss of viability of spores with increased durations of temperature pre-treatment/desiccation/storage (- - ⟶) in *Anabaena fertilissima* and *Anabaenopsis arnoldii*. (B–D) Sequential stages in loss of viability of spores in intact sporulated filaments. Figures depicting the general pattern of germination in sporulated filaments of *A. arnoldii* when (B) undesiccated, (C) desiccated for 48–120 h and (D) desiccated for 240 h.

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