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Review

Survival and Development Strategies of Cyanobacteria through Akinete Formation and Germination in the Life Cycle

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Abstract: Eutrophic freshwater ecosystems are vulnerable to toxin-producing cyanobacteria growth or harmful algal blooms. Cyanobacteria belonging to the Nostocales order form akinetes that are similar to the seeds of vascular plants, which are resting cells surrounded by a thick membrane. They overwinter in sediment and germinate when conditions become favorable, eventually developing into vegetative cells and causing blooms. This review covers the cyanobacterial akinete of the Nostocales order and summarizes the environmental triggers and cellular responses involved in akinete germination and formation based on data from the literature. It also emphasizes the intimate and dynamic relationship that exists between the germination and formation of akinete in the annual life cycle of cyanobacteria. After comparing many published data, it is found that the tolerance ranges for factors affecting both akinete germination and formation do not differ significantly and are broadly consistent with the tolerance ranges for vegetative cell growth. However, the optimal range varies with different species and strains of cyanobacteria. The life cycle of cyanobacteria, as a result of akinete germination and formation, has a seasonal periodicity and spatial connectivity between the water column and the sediment. However, during the summer growing season, intimate coupling between akinete formation and germination can occur in the water column, and this can contribute to high population densities being maintained in the water column. During this time, shallow sediment could also provide suitable conditions for akinete germination, thereby contributing to the establishment of water column populations. The information summarized in this review is expected to help improve our shared understanding of the life cycle of the Nostocales cyanobacteria while also providing insights into the monitoring and management of harmful algal blooms.

Keywords: cyanobacteria; life cycle; akinete; germination; formation; pelagic–benthic coupling

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1. Introduction

Cyanobacteria are known to be the primary source of various toxins and odorous substances in freshwater ecosystems [1,2]. Bloom of cyanobacteria pose a critical threat to water resources management by causing harm to the ecosystem and human health, and thus impairing water use [3–5].

Some cyanobacteria that cause harmful algal blooms have a survival strategy that enables multi-year blooms that allow them to overcome periods of unfavorable growth by forming specialized cells called akinete within their life cycle [6,7]. The akinete-forming cyanobacteria are filamentous cyanobacteria that mostly belong to the Nostocales order, of which the genera *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Nodularia*, *Gloeotrichia*, *Nostoc*, *Nostochopsis*, and *Westiellopsis* are known, while the genera of *Hapalosiphon* and *Stigonema* of the Stigonematales order have also been reported [8,9]. Compared to vegetative cells, akinetes are larger in size [9] and surrounded by a thick cell wall, which can protect them from adverse environmental conditions [10]. Akinetes also contain large amounts of

energy stores and nucleic acids, which allow them to grow quickly through germination when the surrounding environmental conditions are favorable. In this way, akinetes serve as seeds for the initial growth of vegetative cells [11]. Akinetes endow an ecological advantage because they can survive unfavorable growth conditions, and thus, cyanobacteria can produce akinetes to enable perennial blooms [12,13].

The life cycle of akinete-forming cyanobacteria can be divided into vegetative cell and akinete stages [14]. These two stages have ecological significance by bridging the water column and the sediment [15]. Akinetes formed from vegetative cells remain dormant in the sediment when the growth conditions are unfavorable, but when these environmental conditions improve, they germinate and are recruited to the water column to grow into vegetative cells [11,16–18]. Therefore, it is very important to have information on the environmental factors involved in the formation and germination of akinetes and their tolerances to understand the overall life cycle of cyanobacteria, including their death and development, and such information can provide basic guidance for harmful algal bloom management.

Many studies have shown that the formation and germination of akinetes are influenced by a variety of environmental factors, including temperature [7,19,20], light [20,21], nutrients [20,21], dissolved oxygen [7], salinity [19,22], the soil properties of the sedimentary layers depending on depth [7], and bioturbation by invertebrates [23]; of these, there has been very little research examining factors other than temperature, light, and nutrients. Light is known to be a trigger factor that promotes akinete germination and recruitment to the water column [9,11,24], but limiting light can induce akinete formation [25,26]. Different species of cyanobacteria also show varying germination rates depending on light intensity, but within a certain range, light intensity is positively correlated with germination [10,17,24,27]. Akinetes do not seem to germinate under dark conditions [9,21,28,29], but in a few cases, they germinate even in the absence of light [11,30]. Temperature and nutrients are both known to affect the metabolic activity of akinetes, specifically by shortening germination time or inducing akinete formation [31–33]. Different species of cyanobacteria have different optimal temperature and nutrient concentration ranges that affect akinete formation and germination [32–35]. In particular, one might expect nutrients to have different responses in different species, even at the same concentration [36]. Hydrogen ions can affect the germination of akinete in a wide tolerance range, but high germination rates generally occur around neutral and slightly alkaline conditions [27,37,38]. The effect of dissolved oxygen (DO) on the germination of akinete is unclear due to limited studies, but the presence of oxygen may react to facilitate germination [29,39].

To appropriately manage the bloom of harmful cyanobacteria that form akinete, it is necessary to consider the connection between the water column and sediment through akinete [40]. It is also important to understand the life cycle of cyanobacteria by comprehensively considering the formation and germination of akinete [11]. To date, many studies have collected data on the growth and bloom development of cyanobacteria [1,13,30,41], akinete formation [11,25,35,42–44], and germination [11,21,30,45–47], but most such information has been fragmentary. In particular, there has been little research simultaneously considering both akinete formation and germination in the context of a comprehensive life cycle.

This review aims to understand the survival and developmental strategies and life cycle of harmful cyanobacteria belonging to the Nostocales order. To this end, the present review compiles and summarizes information from the existing literature examining cyanobacteria akinete germination and formation in response to various environmental factors and their tolerances. The information in this review is expected to aid our shared understanding of the life cycle of akinete-forming cyanobacteria and provide insights that can be useful in the management of harmful algal blooms.

2. Factors Affecting Akinete Germination and the Ranges of Tolerances

2.1. Temperature

In aquatic ecosystems, temperature is one of the critical environmental factors that drive the seasonal succession of algae [48]. In particular, many eutrophic lakes and streams in temperate climate regions experience cyanobacteria blooms during the summer because such blooms prefer high water temperatures [2]. The germination of akinetes is strongly influenced by temperature. Several experimental studies have shown that the range of temperatures that induce akinete germination is quite wide, where the optimum temperature for the initiation and triggering of germination is species-specific. *Nodularia spumigena* akinetes began to germinate at 15 °C, and the germination rate increased with increasing temperature, with the highest germination rate (80%) seen at 24 °C (experimental condition: temperature 12–24 °C, light 25 μmol $m^{-2}s^{-1}$, BG-11 medium) (Figure 1. ⑨) [20]. Akinetes of *Dolichospermum circinale* germinated over a wide range of water temperatures (12–25 °C) but with very narrow optimal temperature ranges [11]. Park [11] reported that akinetes of *D. circinale* isolated from the sediment of a reservoir started germination at 12 °C and showed a low germination rate (<10%) until 17 °C but then germinated explosively (>80%) at 19–20 °C (experimental condition: temperature 12–25 °C, light 30 μmol m^{-2} s^{-1}, filtered lake water in chamber) (Figure 1. ①). The optimal germination temperature (19–20 °C) coincided with the average reservoir water temperature in June when there was an increased density of vegetative cells, and explosive akinete germination led to the development of vegetative cells in the water column. These results provide important information that can inform the proactive identification and control of *D. circinale* blooms in the field. Other data indicate that *D. circinale* akinetes may have a wider range of germination temperatures (5–38 °C) depending on the characteristics of the water body in which they reside and that temperatures above 20 °C (20–25 °C) are required to trigger germination (Figure 1. ⑤). However, multiple studies have shown that very high temperatures (>30 °C) lead to a tendency for no germination or reduced germination rates [10,19,29]. *Anabaena vaginicola* akinetes have been shown to germinate over a relatively wider temperature range (5–45 °C). The highest germination rate occurred at 25 °C (94%), while it decreased to less than 5% at temperatures > 35 °C (experimental condition: temperature 5–45 °C, light 32.4 μmol $m^{-2}s^{-1}$, Basal medium) [49] (Figure 1. ⑥). Meanwhile, the akinetes of *Cylindrospermopsis raciborskii* have been found to germinate in the range of 15–25 °C, and the optimum germination temperature has been found to be 22–24 °C (Figure 1. ⑩) [50–52].

Taken together, these results suggest that akinetes of some species of cyanobacteria have a similar range of germination and triggering temperatures. On the other hand, the germination of akinetes also seems to be related to the temperature regime of the specific environment(s) in which they live and the temperature tolerances of the cyanobacteria. In Sweden, *Anabaena solitaria* akinetes showed the highest germination rate (40%) at 17 °C (experimental condition: temperature 7–17 °C, light 100 μmol $m^{-2}s^{-1}$, filtered lake water) (Figure 1. ④) [15]. *Anabaena ucrainica* akinetes started germination at 8 °C, with a high germination rate of 70% in the range from 14 to 23 °C (experimental condition: temperature 5–32 °C, light 70 μmol $m^{-2}s^{-1}$, CT medium) (Figure 1. ⑧); the temperature range of germination was 8–32 °C, but the germination rate decreased (<40%) above 26 °C [45]. Unlike other cyanobacteria in the genus *Anabaena*, *Anabaena flos-aquae* akinetes have shown high germination rates at low temperatures (5 °C: 49%, 10 °C: 51%) (experiment condition: temperature 5–30 °C, light 30 μmol m^{-2} s^{-1}, filtered lake water) (Figure 1. ③). However, akinete germination was significantly reduced (<2%) at temperatures above 20 °C. The high germination rate of this species at low temperatures coincided with the water temperature range (<10 °C) during the season (Oct-Nov), during which the cell density of *A. flos-aquae* spiked in the field [30]. By contrast, in India, *Anabaena iyengarii* did not germinate at all at <29 °C, while it predominantly germinated (>40%) at 29–35 °C (experimental condition: temperature 29–41 °C, light 0–40.5 μmol $m^{-2}s^{-1}$, BG-11 medium) [25]. Akinetes of *Nostochopsis lobatus* and *Westiellopsis prolifica* have also shown high germination rates at

high temperatures (29–35 °C: >30%), where the highest germination rates were 66% and 60%, respectively, at 35 °C (Figure 1. ⑬, ⑭) [25].

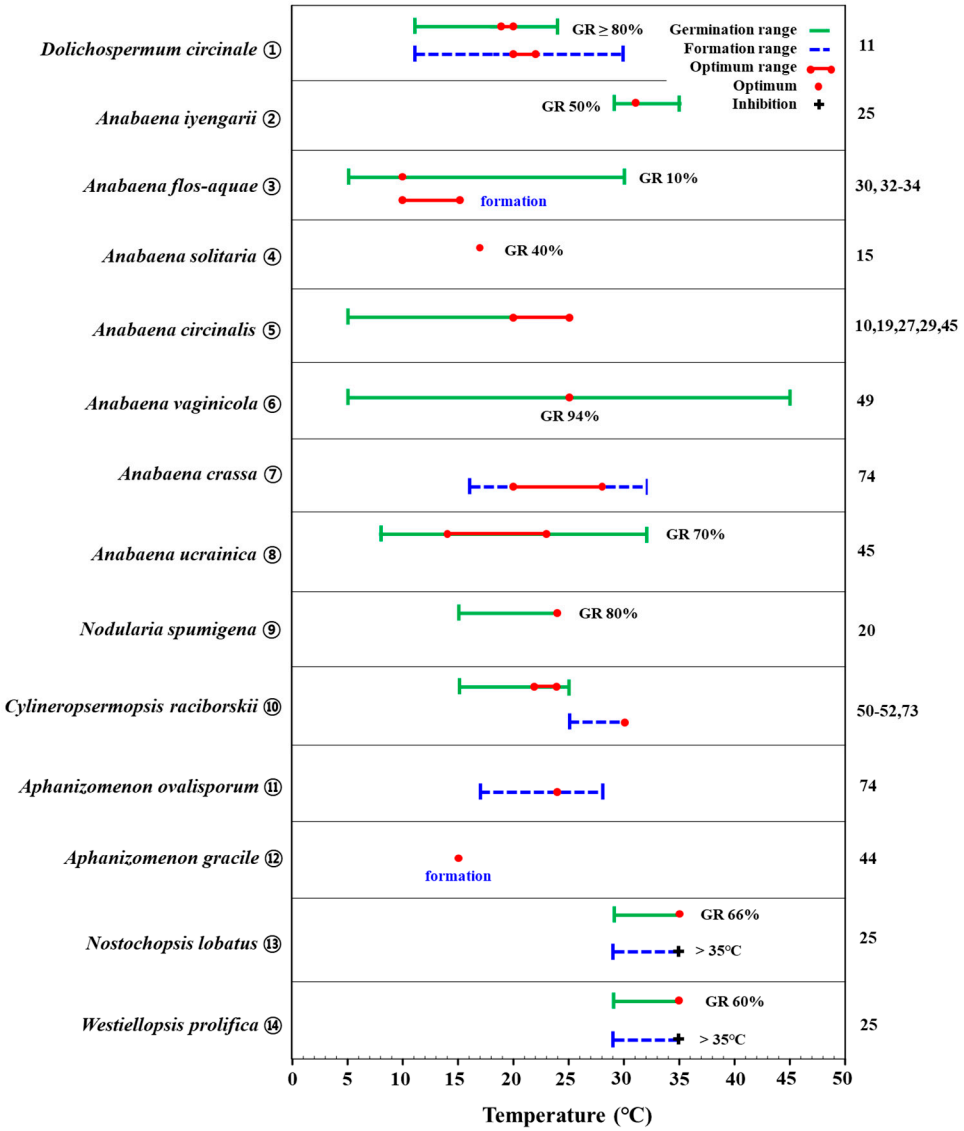

Figure 1. Graphic summary of temperature-dependent akinete germination and formation of cyanobacterial species from the Nostocales order. Numbers appearing on the right column are those in accordance with the list in the references. GR: akinete germination rate.

The literature clearly indicates that temperature is a critical factor in the germination of akinetes. However, temperature tolerances, including the temperature at which germination begins and the optimum temperature for germination, vary among different species of cyanobacteria. Differences in laboratory culture conditions (temperature, light, nutrients, etc.) may affect the germination of akinetes of the target species, but another factor may be the adaptation of the cyanobacteria in the field to the local environment (and the temperature regime in particular) or laboratory culture conditions. For example, the difference between *Anabaena flos-aquae*, which has a high germination rate at low temperatures, and *Anabaena iyengarii*, which has a high germination rate at high temperatures, is understood to reflect differences in water temperature in the environments in which these two species occur [25,30]. *D. circinale* has also been shown to have different germination temperature ranges in several studies [11,19,29]. These results suggest the possibility of the occurrence of ecotypes, by which even the same species shows different germination responses in terms of temperature when inhabiting different environments [53–55]. In conclusion, al-

though the germination of akinetes in cyanobacteria can occur at low temperatures, the optimal germination temperature at which a high proportion of germination is triggered is similar to the temperature range at which vegetative cells grow actively, suggesting that the germination of akinetes and the development of vegetative cells in the aquatic environment may be closely linked in time.

2.2. Light

Prior studies have demonstrated that light is a critical resource that allows akinetes to germinate [20,27,30]. *Anabaena circinalis* has been found to germinate in the light intensity range of 5–100 μmol $m^{-2}s^{-1}$, with a maximum germination light intensity of 30 μmol $m^{-2}s^{-1}$ (Figure 2. ③) [21,29]. According to van Dok and Hart [21], *A. circinalis* akinetes did not germinate in dark conditions without light, and an increase in light intensity in the range of 15–50 μmol $m^{-2}s^{-1}$ did not significantly induce an increase in germination rate, thus showing similar results (germination rate: 17–23%) (experimental condition: temperature 25 °C, light 0–50 μmol $m^{-2}s^{-1}$, ASM-1 medium). Park et al. [29] also showed that akinetes of *A. circinalis* did not germinate under dark conditions, with a germination rate of 45% at 5–10 μmol $m^{-2}s^{-1}$ light conditions, and the highest germination rate of 60 % at 30 μmol $m^{-2}s^{-1}$. On the other hand, at light intensity exceeding 50 μmol $m^{-2}s^{-1}$, the germination rate decreased (10%) (experimental condition: temperature 25 °C, light 0–100 μmol $m^{-2}s^{-1}$, filtered lake water). *Anabaena cylindrica* akinetes also germinated in a wide light intensity range of 2–60 μmol $m^{-2}s^{-1}$ (experimental condition: temperature 27 °C, light 2–60 μmol $m^{-2}s^{-1}$, Detmer medium) (Figure 2. ④) [27].

Several studies have demonstrated that light is an essential resource for akinete germination, but some authors have also observed germination in the dark. However, the observed effect has been very small. In Park [11], *Dolichospermum circinale* akinetes were shown to be germinated in the dark, albeit very weakly (germination rate: 3%) (experimental condition: temperature 20 °C, light 0–100 μmol $m^{-2}s^{-1}$) (Figure 2. ①). Further, Kim et al. [30] showed that *Anabaena flos-aquae* germinated under dark conditions (germination rate: 4%) (Figure 2. ⑤). As light is an essential resource for photosynthesis, then even if germination can occur under dark conditions, such conditions still cannot promote vegetative cell growth, so akinete germination under dark conditions observed in the above studies may not be a common phenomenon, particularly in the conditions that are common in the field. The observed positive correlation between chlorophyll-*a* concentration and germination rate in akinetes of *Anabaena circinalis* suggests that their germination rate is related to photosynthesis [10].

In general, the light intensity required for akinete germination is not high [20,21,25], but the range of light intensities for germination and the optimum light intensity varies among different species of cyanobacteria. Further, in certain light intensity ranges, akinete germination is promoted by increasing light intensity [9,24]. *Anabaena flos-aquae* akinetes were found to germinate at light intensity 0–30 μmol $m^{-2}s^{-1}$ and showed a high germination rate (>53%) at 5–15 μmol $m^{-2}s^{-1}$ (experimental condition: temperature 10 °C, light 0–30 μmol $m^{-2}s^{-1}$, filtered lake water) [30]. *Anabaena iyengarii* akinetes also germinated under low light conditions (experimental condition: temperature 29–41 °C, light 0–40.5 μmol $m^{-2}s^{-1}$) [25]. They germinated more than 20% in the range of light intensity 4.05–27 μmol $m^{-2}s^{-1}$, while they showed the maximum germination rate (38%) at light intensity 4.05 μmol $m^{-2}s^{-1}$. On the other hand, no germination of *A. iyengarii* akinetes occurred under very low and high light intensity (0–1.35 μmol $m^{-2}s^{-1}$ and 40.5 μmol $m^{-2}s^{-1}$, respectively), nor did *Nostochopsis lobatus* and *Westiellopsis prolifica* akinetes germinate under the same experimental conditions (Figure 2. ⑩,⑪). For *N. lobatus*, the akinete germination rate increased with increasing light intensity within the range of 4.05–27 μmol $m^{-2}s^{-1}$, and it was highest (38%) at 27 μmol $m^{-2}s^{-1}$. *W. prolifica* akinete started to germinate at 4.05 μmol $m^{-2}s^{-1}$ (30% germination rate), followed by germination rates of 14% at 6.75 μmol m^{-2} s^{-1} and 28 % at 27 μmol $m^{-2}s^{-1}$ [25]. On the other hand, *Nodularia spumigena* germinated at a rate of 29% under very low light conditions of 0.5 μmol m^{-2} s^{-1}, and the germination rate increased with increasing light intensity (experimen-

tal condition: temperature 21 °C, light 0–99 µmol m^{-2} s^{-1}) (Figure 2.⑥) [20]. This species did not germinate under dark conditions, with a germination rate of 51% at 9 µmol m s^{-2-1} and a rate of more than 80% at 80 µmol $m^{-2}s^{-1}$. Notably, germination was triggered at low light levels, ranging from 0.5 to 9 µmol $m^{-2}s^{-1}$, after which it continuously up to 80 µmol $m^{-2}s^{-1}$.

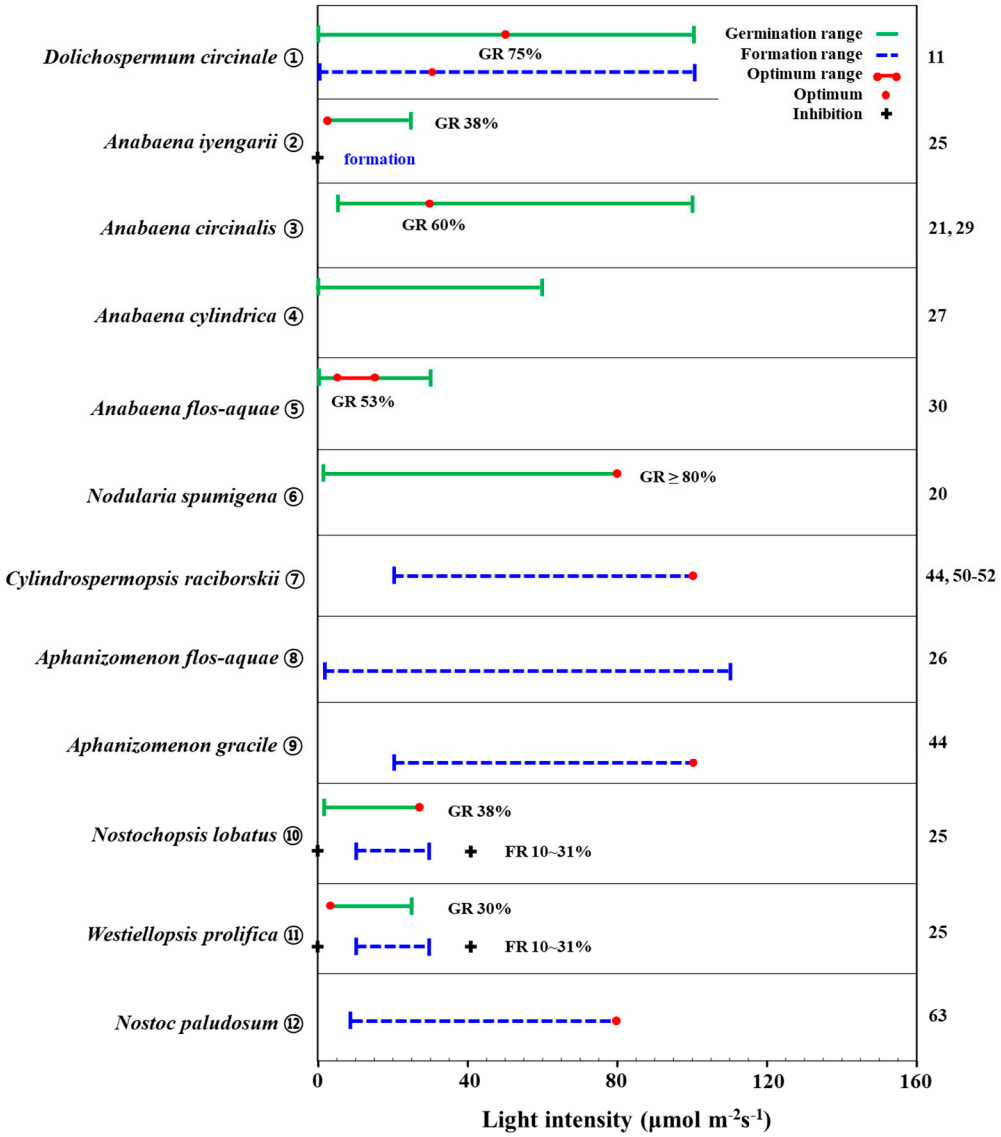

Figure 2. Graphic summary of light-dependent akinete germination and formation of some cyanobacterial species from the Nostocales order. Numbers appearing on the right column are those in accordance with the list in the references. GR: akinete germination rate. FR: akinete formation rate.

Several previous studies have shown that akinete germination occurs over a wide range of light intensities, although with generally higher germination rates at lower light intensities than the corresponding rates at higher light intensities. This suggests that akinetes do not require high light levels to germinate. Although some cyanobacterial akinetes have been shown to germinate in the dark, albeit to a very small extent, in most experiments, akinetes did not germinate in the dark. Given that the germination of akinetes in the field begins in the sediment, which is subject to dilution and extinction of light through the water column, it is ecologically plausible to assume that germination occurs under low light conditions [56]. Further, within the range of low light intensities, an increase in light intensity is linked to an increase in photosynthesis in germinated cells, which may facilitate akinete germination. Compared to water temperature, changes in light

intensity are relatively less seasonal, so the effect of light on akinete germination in the field is likely to be more dependent on spatial differences (water column versus sediment) than seasonal differences. In particular, for akinetes that are distributed in shallow sediments, the availability of light is likely to initiate and trigger germination [57].

2.3. Nutrients

Unlike external stimuli, such as temperature and light, nutrients can potentially affect akinete germination by determining the physiological state inside the akinete cell; i.e., nutrients are involved in the synthesis of energy materials and nucleic acids that akinetes need to activate germination and cell division [58,59]. Several studies have demonstrated the potential impact of nitrogen and phosphorus on akinete germination [20,21,37,49]. It has also been shown that different types of nutrients affect germination in unique ways and that the concentration of nutrients necessary to trigger germination differs not only among different species but also within the same species [11,36].

Two studies on *Nodularia spumigena* showed the existence of different dose-response relationships between akinete germination and nutrient (nitrogen and phosphorus) concentrations (Figure 3. ⑧; Table 1) [20,46]. In Huber [20], *N. spumigena* akinetes germinated more than 80% evenly in a wide concentration range (0–54.3 mgL^{-1}) when nitrate was added, regardless of the concentration difference, but this did not occur when ammonium was added (0.14–2.28 mgL^{-1}), as the germination rate, in that case, was highest (>80%) at 0.14 mgL^{-1}, and germination was inhibited at higher concentrations (experimental condition: temperature 21 °C, light 25 μmol $m^{-2}s^{-1}$, N and P addition in BG-11 medium). On the other hand, overall, more than 60% of akinetes germinated under the conditions of phosphorus addition (0.113–28.212 mgL^{-1}), but they did not germinate at all when phosphorus was deficient. Huber [20] showed that nitrate had no effect on germination and that a very low phosphorus concentration was required for germination. However, Myers et al. [46] showed that *N. spumigena* akinete germination rates were positively correlated with both nitrate and phosphorus concentrations (experimental condition: temperature 21 °C, light 40, 100 μmol $m^{-2}s^{-1}$, N and P addition in MLA medium). The addition of phosphorus in the concentration range of 0–2.5 mgL^{-1} showed a maximum germination rate of 20% at a concentration of 2.5 mgL^{-1}. The overall germination rate as a function of phosphorus concentration was shown to be affected by light intensity, with the germination rates observed at 40 μmol $m^{-2}s^{-1}$ being higher than those observed at 100 μmol $m^{-2}s^{-1}$. In the range of nitrate concentrations from 0 to 3 mgL^{-1}, the highest germination rate was 25% at 3 mg/L, and contrary to the case of phosphorus, the germination rate was higher at 100 μmol $m^{-2}s^{-1}$.

The results of some prior studies indicated that nitrogen has an unclear effect on akinete germination (Figure 3. ③, ④; Table 1) [21,27]. In Van Dok and Hart [21], *Anabaena circinalis* akinetes germinated at a high rate even without nitrogen addition. The germination rate was increased with increasing nitrate concentration; meanwhile, ammonium addition inhibited akinete germination. On the other hand, the germination rate increased by more than three times with the addition of phosphorus (experiment condition: temperature 25 °C, light 30 μmol $m^{-2}s^{-1}$, N and P addition in ASM-1 medium). In Yamamoto [27], the addition of nitrate had no effect on *A. cylindrica* akinete germination; instead, organic carbon (acetate) accelerated germination. By contrast, Rai and Pandey [49] reported that nitrate had a greater effect on akinete germination than phosphorus, based on the results indicating that *Anabaena vaginicola* showed a high germination rate of 96% in nitrate-supplemented conditions whereas it showed a germination rate of 43% in phosphorus-deficient conditions (experimental condition: temperature 25 °C, light 32.4 μmol $m^{-2}s^{-1}$, basal medium) (Table 1).

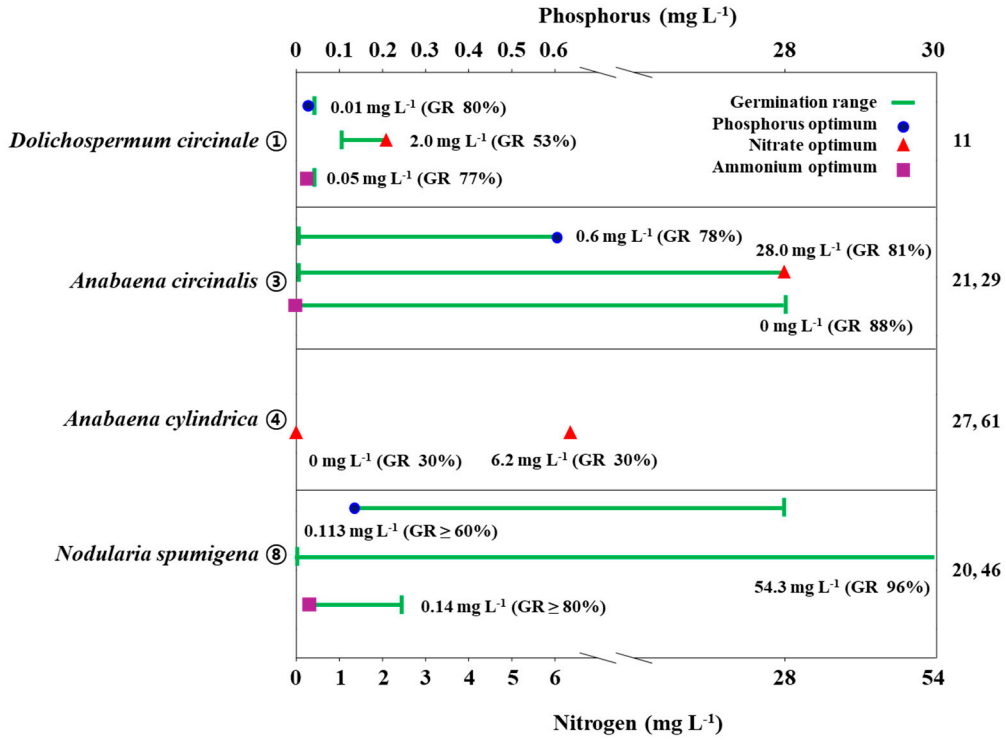

Figure 3. Graphic summary of nutrient-dependent akinete germination and formation of some cyanobacterial species from the Nostocales order. Numbers appearing on the right column are those in accordance with the list in the references. GR: akinete germination rate.

On the other hand, both nitrogen and phosphorus appear to have a synergistic effect on akinete germination (Figure 3. ③; Table 1) [21,29]. Park et al. [29] found that *Anabaena circinalis* akinete germination was highest (55%) in conditions involving the addition of both N and P, while the germination rate was lowest (10%) in conditions where both of them were deficient. However, when N and P were treated separately, nitrogen induced a higher germination rate (50%) than phosphorus (<25%) (experimental condition: temperature 25 °C, light 30 µmol $m^{-2}s^{-1}$, -N-P, +N-P, -N+P, +N+P in CB medium). The same results were found in *Anabaena iyengarii* akinetes [59]. Specifically, Agrawal and Misra [37] reported that germination was highest (50%) in conditions where both nitrate and phosphorus were added; meanwhile, in conditions where nitrate and phosphorus were deficient, germination decreased to 30% and 40%, respectively. The germination rate was lowest (20%) in the condition where both N and P were deficient (experiment condition: temperature 22 °C, light 40 µmol $m^{-2}s^{-1}$, -N-P, +N-P, -N+P, +N+P in basal medium) (Table 1). In Park's [11] study, where nitrate and ammonium were used as nitrogen sources simultaneously, the main nutrients of *Dolichospermum circinale* akinete germination were ammonium and phosphorus, while in the nitrate condition, more than 80% of the akinetes germinated in the P-only addition regardless of N addition, thus showing the synergistic effect of N and P (experimental condition: temperature 20 °C, light 30 µmol $m^{-2}s^{-1}$, -N-P, +N-P, -N+P, +N+P in CB medium) (Figure 3. ①; Table 1). On the other hand, in the ammonium addition condition, the germination rate was highest (91%) in the [+N-P] condition, while P had an almost insignificant effect. Based on the experimental results, the optimal concentration range for *D. circinale* akinete germination was suggested to be NO_3-N 1–3 mgL^{-1}, NH_4-N 0.05–0.2 mgL^{-1}, and PO_4-P 0.005–0.5 mgL^{-1} [11]. *Westiellopsis prolifica* akinetes were germinated at a more than five-fold increase under conditions with added nitrogen or phosphorus compared to nitrate or phosphorus deficiency [60]. The germination rate was highest at 58% under conditions where both nutrients were added simultaneously [37]. This species germinated 35% in nitrate deficiency and 26% in phosphorus deficiency, with the lowest germination rate of 20% in conditions where both were absent. Similarly,

Nostochopsis lobatus showed similar germination rates (54%) to *A. iyengarii* and *W. prolifica* in conditions where both nitrate and phosphorus were added (Table 1) [37]. However, their germination rates in nitrate-deficient and phosphorus-deficient conditions were similar, at respective values of 38% and 35%, while they showed a germination rate of 24% in conditions lacking both nutrients.

Altogether, the literature on the relationship between nutrients and akinete germination suggests that nitrogen and phosphorus both play important roles in germination. However, the effects of nitrogen and phosphorus are different for akinetes of different species of cyanobacteria, and they are also different for the same species of cyanobacteria (Figure 3; Table 1). Nitrogen and phosphorus also appear to be synergistic in increasing akinete germination rates. The nutrient dose responses to germination were shown to vary among species, and even within the same species, and for nitrogen, the contribution of different nitrogen sources was different, with nitrate having extreme effects. In some cases, germination occurred even in the absence of nitrate, while in others, germination increased with higher nitrate concentration. However, in many cases, ammonium had a greater impact on increasing germination rates than nitrate.

These conflicting results across studies may be attributable to several factors, including different experimental conditions (e.g., differences in medium, field isolates, or laboratory cultures, differences in physiological status, differences in nutrient concentrations treated, differences in environmental conditions other than nutrients, etc.). They may also be due to the different physiological effects of nitrogen and phosphorus on akinete germination and growth for cyanobacterial species. In the field, nutrients may not be a critical factor limiting akinete germination, as suggested by the fact that the germination of akinetes has been observed under experimental conditions even without the addition of nutrients [7]. This may be because, within aquatic ecosystems, akinetes are present in the sediment or at the water–sediment interface, where the nutrient concentrations are typically high. Moreover, akinetes contain high concentrations of nitrogen and phosphorus inside their cells. Under these circumstances, it is unlikely that additional nutrients will be needed. However, in situations where akinete germination is already underway, the supply of additional nutrients will induce an increase in the germination rate and contribute to the development of vegetative cells. Therefore, nutrients are likely to play a greater role in the progression of germination to vegetative cells than they do in influencing the initiation of germination.

Table 1. Literature summary of nutrients effects on cyanobacterial akinete germination and formation.

No	Cyanobacteria Species	Effects of N and P on AKINETE	Ref
①	*Dolichospermum circinale*	With nitrate: Germination rate (GR) > 80% in (-N or +N)+P condition (Little effect of nitrate) With ammonium: GR 91% in +N-P condition (Little effect of P) The highest akinete formation (420 akinetes/g) in +N+P ($N=NH_4$-N) condition	[11]
②	*Anabaena iyengarii*	GR 50% in +N+P ($N=NO_3$-N); GR 20% in -N-P ($N=NO_3$-N) Formation rate (FR) 6% in +N+P condition	[37]
③	*Anabaena circinalis*	GR 55% in +N+P; GR 50% in +N-P; GR 25% in -N+P; GR 10% in -N-P Akinete formation in P (0.06mg/L) addition; no formation without N	[21,29]
④	*Anabaena cylindrica*	No akinete formation in the absence of N	[61]
⑤	*Anabaena vaginicola*	GR 96% in NO_3-N addition; GR 43% in P deprivation	[49]
⑥	*Anabaena lemmermannii*	Akinete formation in the absence of P	[43]
⑦	*Anabaena crassa*	No formation when depriving both N and P	[34]
⑧	*Nodularia spumigena*	High germination (>80%) under broad range of NO_3-N (0–54.3 mg/L)	[20]
⑨	*Cylindrospermopsis raciborskii*	FR increased with increasing P conc. Maximum akinete formation (2310 akinetes/mL) in 70 μgP/L	[62]

Table 1. *Cont.*

No	Cyanobacteria Species	Effects of N and P on AKINETE	Ref
⑩	*Nostoc palusodum*	Akinete formation in the absence of P	[63]
⑪	*Nostochopsis lobatus*	GR 54% in +N+P; GR 38% in -N+P; GR 35% in +N-P; GR 24% in -N-P FR 55% in +N+P	[37]
⑫	*Westiellopsis prolifica*	GR 58% in +N+P; GR 35% in -N+P; GR 26% in +N-P; GR 20% in -N-P FR 70% in +N+P	[37]

2.4. Hydrogen Ion (H^+) Concentration

Several studies have demonstrated that hydrogen ion concentration is an environmental factor that substantially affects both akinete germination and vegetative cell growth [37,64]. The effect of pH on akinete germination has been reported in several species of the genus *Anabaena*. *Fischerella muscicola* germinated in the pH range from 6 to 10 and did not germinate at all in acidic conditions (pH < 5) (Figure 4.①). Moreover, the germination rate increased with decreasing H^+ concentration, with the maximum germination rate at pH 9 (68%), and the germination rate decreased rapidly (<50%) at pH 10 and above (experimental condition: temperature 25 °C, light 50 µmol $m^{-2}s^{-1}$, N-free chu medium, pH 5–10) [65]. In the range of pH 6–11, *Anabaenopsis arnoldii* akinetes had the highest germination rate at pH 7 (64%) and maintained a high germination rate up to pH 8.5 (58%). However, the germination rate decreased at pH 9 and above (experimental condition: temperature 28 °C, light 32.4 µmol $m^{-2}s^{-1}$, SSM medium, pH 4.5–11) [38].

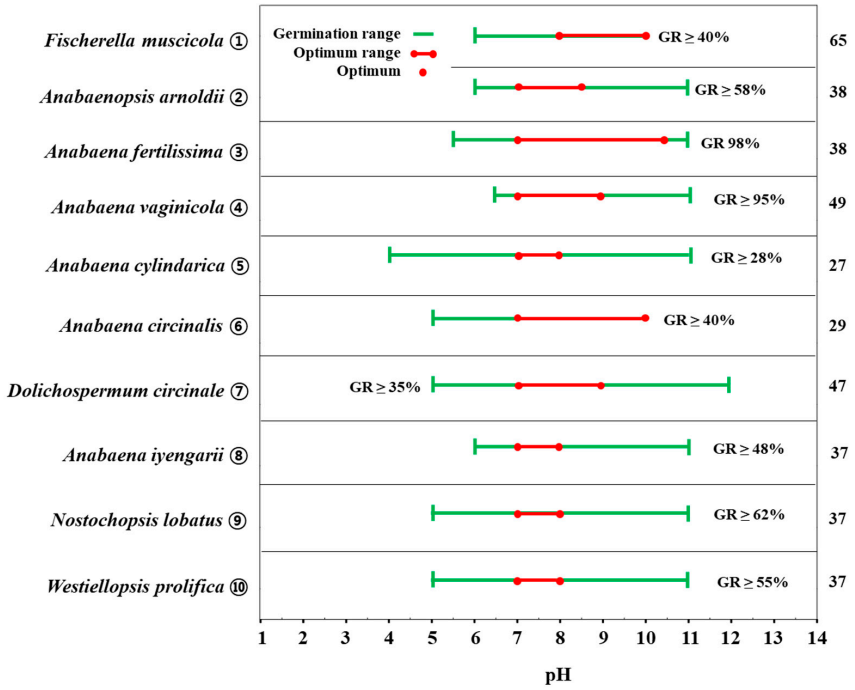

Figure 4. Graphic summary of pH-dependent akinete germination of some cyanobacterial species from the Nostocales order. Numbers appearing on the right column are those in accordance with the list in the references. GR: akinete germination rate.

By contrast, *Anabaena fertilissima* germinated evenly over a wide range of pH from 5.5 to 11 (Figure 4. ③). Almost all akinetes germinated at pH 7–10.5 (98%), with 75% germination observed even at pH 11. Notably, this species was found to germinate in all acidic, neutral, and alkaline conditions (experimental condition: temperature 28 °C, light 32.4 µmol $m^{-2}s^{-1}$, SSM medium, pH 4.5–11) [38]. *Anabaena vaginicola* started germination at pH 6 (23%), and the range in which it showed maximum germination was

pH 7–9 (>95%). However, akinetes did not germinate under conditions below pH 5.5 and above pH 11 (experimental condition: temperature 25 °C, light 32.4 µmol $m^{-2}s^{-1}$, Basal medium, pH 5–12) (Figure 4. ④) [49]. Unlike other cyanobacteria in the genus *Anabaena*, *Anabaena cylindrica* akinetes even germinated under acidic conditions of pH 4. *A. cylindrica* akinete germinated in a very wide range from pH 4 (<5%) to pH 11 (<20%), while its germination rate was highest (28–30%) at pH 7–8. However, germination decreased below pH 6 and above pH 10 (experimental condition: temperature 27 °C, light 67.5 µmol $m^{-2}s^{-1}$, Detmer medium, pH 2–12) (Figure 4. ⑤) [27].

The decrease in akinete germination under acidic and alkaline conditions appears to be related to the destruction of akinetes under strong acidic and strong alkaline conditions. Park et al. [29] observed the destruction of the akinete cell wall in *Anabaena circinalis* at pH 5–6 (germination rate: 10%) and pH 11–12 (0%). High germination rates were found in the ranges of pH 7–8 (55%) and pH 9–10 (40–45%) (experimental condition: temperature 25 °C, light 30 µmol $m^{-2}s^{-1}$, CB medium, pH 5–12) (Figure 4. ⑥). Very high or low pH can alter the permeability of the cell membrane, which affects ion uptake and may also lead to a loss of soluble metabolites [66,67]. On the other hand, in Kwon et al. [47], *Dolichospermum circinale* akinetes were shown to germinate in strong alkaline conditions of pH 12, albeit weakly (<10%) (experimental condition: temperature 25 °C, light 30 µmol $m^{-2}s^{-1}$, CB medium, pH 5–12) (Figure 4. ⑦). However, aligning with the results of other studies, the range in which maximum germination occurred was found to be pH 7–9, with 35% germination at pH 7, 70% germination at pH 8, and 50% germination at pH 9. *Anabaena iyengarii* akinetes germinated in the pH 6–11 range. The highest germination rate occurred at pH 7–8 (48–50%), and the germination rate decreased by more than 40% as the pH increased from 8 to 11 (experiment condition: temperature 22 °C, light 40 µmol $m^{-2}s^{-1}$, basal medium, pH 4–11) (Figure 4. ⑧) [37].

Nostochopsis lobatus akinetes have also been shown to germinate at the highest rate (>62%) at pH 7–8 (Figure 4. ⑨) [37]. However, they showed low germination rates of < 30% at acidic conditions, pH 5–6, and weakly basic conditions, pH 9–11. Similarly, *Westiellopsis prolifica* exhibited the highest germination rate (>55%) at pH 7–8. At pH 5, the germination rate was 5%, and at pH >9, the germination rate started to decrease, with the lowest germination rate (6%) observed at pH 11 (Figure 4. ⑩) [37].

Most prior studies have shown that the pH tolerance for the germination of cyanobacteria akinetes broadly ranges from acidic to alkaline but that the most favored range for germination is neutral to slightly alkaline and does not seem to vary much among species. In some experiments, species have been reported to germinate in strongly acidic or strongly alkaline conditions, such as pH 4 and pH 12, but at very low or zero rates (0-< 10%). Since akinete needs to grow into a vegetative cell after germination, it is not surprising that the pH conditions for germination and cell growth are almost identical [29,37,68].

2.5. Dissolved Oxygen (DO), Salinity, and Sediment Disturbance (Mixing and Turnover)

The tolerance of akinetes to DO is not well understood, and there have been limited DO studies involving germination. This may be attributable to the fact that, in the field, akinetes are buried in the sediment, which makes it difficult to determine the direct effects of oxygen. Kim et al. [30] measured *Anabaena flos-aquae* akinete germination and DO concentration in the sediment of a reservoir; the reservoir hypolimnion was seen to remain aerobic (7–17 mg O_2L^{-1}) during the sampling period, and there was no correlation observed between akinete germination and DO concentration. However, Fay et al. [10] showed that, for the akinete of *Anabaena circinalis*, after germination at low light levels, the first response was oxygen uptake for respiration. This suggests that DO may not be the primary factor that induces germination but that it is associated with increased cellular activity for energy production (photosynthesis) during the early stages of germination [10]. However, low oxygen concentrations may increase akinete viability in the long term by reducing the energy required for respiration [40]. On the other hand, DO has been shown to be essential for akinete germination in *Anabaena cylindrica* and *Nostoc PCC 7524* [27,39], and it

showed a five-fold higher germination rate under aerobic conditions compared to anaerobic conditions [27].

Several prior studies have shown that different species of cyanobacteria appear to have different salinity tolerances for akinete germination. *Nodularia spumigena*, a brackish-water blue-green alga, showed a decreased germination rate of akinetes in response to the addition of both low concentrations [69] and high concentrations [20] of sodium chloride. Silveira and Odebrecht [70] showed that salinity had a greater effect on *N. spumigena* akinete germination compared to temperature. This species showed that akinete germination was highest at salinities of 7 and 15 ppm, while it was lowest at 1 ppm. Myers et al. [46] also found high germination rates between salinities 5 and 15. These results indicate that fluctuations in salinity in brackish-water conditions significantly affect the germination of this species. Baker and Bellifemine [19] showed that *Anabaena circinalis* is tolerant to moderate salinities. Increasing salinity to 2.5 gL^{-1} increased akinete germination to 27%, with a sharp decrease at >5 gL^{-1} and no germination at the concentration of 10 gL^{-1}.

Several studies support the hypothesis that the mixing of water and sediment plays an important role in the formation of blooms of akinete-producing cyanobacteria. This hypothesis is supported by the results of higher recruitment rates of vegetative cells in shallower sediment, i.e., the shallow littoral zone, which tends to be more susceptible to disturbance than deeper sediment [22,40,57]. Moreover, data from several studies have shown that shallow sediments are important seed banks or inoculation sites for akinetes. In *Gleotrichia echinulate*, recruitment from the sediment bed to the water column via akinete germination was found to be significantly enhanced by mixing the sediment bed through bioturbation or physical processes [23,57]. In Lake Kinneret, the sediment in the littoral zone was found to be easily resuspended by wind-driven waves, which affected the akinete germination and recruitment of *Aphanizomenon ovalisporum* into the water column [71]. Therefore, it is possible that the littoral zone of shallow lakes, shallow marshes, and deep lakes represent a conducive environment for akinete germination because the mixing of sediments and continuous resuspension from the sediment into the water column can expose akinetes to the appropriate environment (e.g., light and oxygen) for germination.

3. Factors Affecting Akinete Formation and the Range of Tolerances

3.1. Temperature

Temperature has previously been identified as a major environmental factor in the formation of akinetes in cyanobacteria [34,72]. In general, a decrease in the water temperature leads to unfavorable conditions for vegetative cell growth and could, therefore, induce akinete formation [33,35,44]. Just as the tolerance range of cyanobacteria vegetative cell growth to temperature is quite wide, it is possible that akinete formation could occur over a wide range of temperatures. However, the optimal temperature range that promotes akinete formation may be species-specific [25,34,73]. On the other hand, the stress that triggers akinete formation can also be affected by the degree of temperature fluctuation. Moore et al. [35] reported that, in experiments with *Cylindrospermopsis raciborskii*, a higher frequency of temperature fluctuations increased akinete production, with multiple 10 °C (25 to 15 °C) temperature changes resulting in the highest formation rates. These temperature-scale differences may be caused by seasonal water temperature fluctuations in the field, but it is also possible for them to occur within a day, given the differences between day and night and water depth.

Since akinetes are also formed during normal vegetative cell division, akinetes can be produced within the temperature range in which vegetative cell growth is active [11,74]. Previously collected data have indicated that several cyanobacteria show high akinete formation rates in the 20–30 °C range. In *Dolichospermum circinale*, *Anabaena crassa*, *Cylindrospermopsis raciborskii*, and *Aphanizomenon ovalisporum*, the temperature range of akinete formation was very wide, ranging from a low of 12 °C to a high of 32 °C, but the optimum formation temperature was generally present at 20–30 °C. According to Park [11], *D. circinale* produced a low density of akinetes (<10 akinetes g^{-1}) at 12–15 °C, while the den-

sity increased rapidly at 20–22 °C (Figure 1. ①). Further, at >22 °C, the akinete formation was greatly inhibited, while at 30 °C, there was almost no akinete formation (experimental condition: temperature 12–30 °C, light 30 μmol $m^{-2}s^{-1}$, filtered lake water). *A. crassa* and *A. ovalisporum* formed akinetes in the respective ranges from 16 to 32 °C and 16 to 28 °C, with the highest formation rate at 24 °C (4×10^4 akinetes L^{-1} and 6.5×10^3 akinetes L^{-1}, respectively) [74]. The akinete formation rate of *A. crassa* also increased with increasing temperature, showing a high formation rate up to 28 °C, and it was inhibited at >28 °C (experimental condition: temperature 16–32 °C, light 35 μmol $m^{-2}s^{-1}$, BG-11 medium) (Figure 1. ⑦). *A. ovalisporum* formed akinetes at a high density from 16 °C to 24 °C, and formation was inhibited at >24 °C (Figure 1. ⑪) [74]. On the other hand, in Yamamoto and Shiah [73], *Cylindrospermopsis raciborskii* formed akinetes in the range of 24–30 °C, with a peak at 30 °C (experimental condition: temperature 18–30 °C, light 60 μmol $m^{-2}s^{-1}$, C medium) (Figure 1. ⑩). The result showing that the temperature at which maximum akinete formation occurs coincides with the temperature range of active vegetative cell growth and blooms suggests that akinetes are continuously formed during the growth of vegetative cells in situ, which in turn raises a question about the role that mass-produced akinetes play in the water column (refer Section 4).

On the other hand, *Anabaena flos-aquae* and *Aphanizomenon gracile* formed akinetes at relatively low temperatures compared to other cyanobacteria from the Nostocales order (Figure 1. ③, ⑫). *A. flos-aquae* formed akinetes at 10–15 °C (experimental condition: temperature 10–35 °C, light 40 μmol $m^{-2}s^{-1}$, CT medium) [34]. Meanwhile, *A. gracile* formed akinetes when the temperature decreased below 15 °C (experimental condition: temperature 15 and 20 °C, light 20–350 μmol $m^{-2}s^{-1}$, Z8 medium) [44]. By contrast, *Nostochopsis lobatus* and *Westiellopsis prolifica* formed akinetes at high temperatures (29–35 °C), but akinete formation was inhibited at temperatures above 35 °C (experimental condition: temperature 29–41 °C, light 0–40.5 μmol $m^{-2}s^{-1}$, BG-11 medium) (Figure 1. ⑬, ⑭) [25].

3.2. Light

Light is a factor affecting akinete formation, and there have been several prior studies examining light limitation [11,16,17,24,75–77]. The tolerance of light intensity to akinete formation varies among species [25,26,44]. Under extreme light conditions (e.g., darkness or very strong light), vegetative cell growth tends to cease, and akinete formation does not occur [25,26].

In Agrawal and Singh [25], *Nostochopsis spumigena* and *Westiellopsis prolifica* showed akinete formation rates of 10–31% under 0.004–27 μmol $m^{-2}s^{-1}$ conditions, and they did not form akinetes dark conditions with no light or 40.5 μmol $m^{-2}s^{-1}$ (experimental condition: temperature 29–41 °C, light 0–40.5 μmol $m^{-2}s^{-1}$, BG-11 medium) (Figure 2. ②, ⑩, ⑪). Rother and Fay [26] showed that *Aphanizomenon flos-aquae* did not form akinetes under high light conditions (>108 μmol $m^{-2}s^{-1}$), and akinete formation was also inhibited under low light conditions of 1.35 μmol $m^{-2}s^{-1}$ (Figure 2. ⑧). No akinetes were formed under UV light conditions either. All cases showing no or very low akinete formation were consistent with arrested or delayed vegetative cell growth [26].

In the growth chamber experiments that were designed to include both water and the sediment parts, dormant sporulation of *Dolichospermum circinale* exhibited a wide tolerance range to light intensity (0–100 μmol $m^{-2}s^{-1}$) [11]. Within the range of 0–30 μmol $m^{-2}s^{-1}$, the rate of akinete formation was found to increase with increasing light intensity, which was consistent with an increase in the number of vegetative cells. Under light conditions of 0–5 μmol $m^{-2}s^{-1}$, the density of akinetes was very low (13 akinetes g^{-1}), and the most (700 akinetes g^{-1}) akinetes were formed at 30 μmol $m^{-2}s^{-1}$, and this density decreased again at 50–100 μmol $m^{-2}s^{-1}$ (160–260 akinetes g^{-1}) (experimental condition: temperature 20 °C, light 0–100 μmol $m^{-2}s^{-1}$, filtered lake water) (Figure 2. ①) [11].

On the other hand, *Nostoc paludosum* promoted akinete formation under very low light conditions (experimental condition: temperature 25 °C, light 60, 30, 8.1 μmol $m^{-2}s^{-1}$,

AA medium) (Figure 2. ⑫) [63]. Reducing the light intensity from 60 μmol m s^{-2-1} to 8.1 μmol $m^{-2}s^{-1}$ induced a 50-fold increase in akinete formation, whereas vegetative cell growth showed the opposite trend. Moreover, akinetes formed at high light intensity were significantly smaller in size [63]. Consistent with the hypothesis suggesting that light restriction induces akinete formation, studies have reported that self-shading by a high density of cells during cyanobacteria bloom leads to an explosion of akinetes in situ [72] and experimental culture conditions [11].

The akinete formation of *Cylindrospermopsis raciborskii* and *Aphanizomenon gracile* in response to light intensity did not show consistent patterns [44]. When three strains of each of these two species were cultured in the light intensity range from 20 to 350 μmol $m^{-2}s^{-1}$, light intensity had different effects on akinete formation (expressed as the ratio of akinete biovolume to total cell biovolume) between the two species as well as between temperatures. In two strains of *Cylindrospermopsis raciborskii*, the proportion of akinetes increased with increasing light intensity up to 300 μmol $m^{-2}s^{-1}$ under 20 °C conditions, while it showed no clear pattern at 15 °C. The two strains of *Aphanizomenon gracile* showed a decrease in the percentage of akinetes with increasing light at 15 °C, but at 20 °C, the percentage of akinetes was very low within the entire range of experimental light. Consequently, in this study, temperature rather than light had a greater effect on the formation of akinetes of *Cylindrospermopsis raciborskii* and *Aphanizomenon gracile* (experimental condition: temperature 15, 20 °C, light 20–350 μmol $m^{-2}s^{-1}$, 8-medium) (Figure 2. ⑦, ⑨) [44]. Further, compared to high continuous light conditions, *Aphanizomenon ovalisporum* showed delayed akinete formation under low continuous light conditions, along with a reduction in the total number of akinetes formed [74].

In addition to the effects of light intensity, spectral quality might also potentially affect akinete formation, depending on the species and strains of cyanobacteria. Thompson et al. [78] showed that blue light significantly reduced *Anabaena circinalis* akinete formation and that the peak rate of akinete formation per vegetative cell was approximately 3000 times higher when treated with red irradiance than it was when treated with blue irradiance under the same light intensity of 40 μmol $m^{-2}s^{-1}$. This suggests that photochemically usable radiation may be involved in akinete formation [76]. Moreover, in the light intensity range from 20 to 350 μmol $m^{-2}s^{-1}$, neither *Cylindrospermopsis raciborskii* nor *Aphanizomenon gracile* showed a consistent pattern between akinete formation and light intensity [44].

3.3. Nutrients

It has long been known that nutrient limitation affects cyanobacteria growth and is a potential factor in akinete formation [43,63,79,80]. However, some researchers have reported that nutrient deficiencies do not necessarily induce akinete formation [34,61,76]. Meanwhile, in some cyanobacteria, the supply of nutrients has been shown to promote akinete formation [11,37,72].

In *Anabaena lemmermannii* and *Nostoc palusodum*, akinete formation was associated with phosphorus. In *A. lemmermannii*, the density of vegetative cells was very low when phosphorus was deficient, but the density of akinetes was high (Table 1) [43]. *N. paludosum* had the highest akinete density in the phosphorus-deficient experimental group (experimental condition: temperature 25 °C, light 120 μmol $m^{-2}s^{-1}$, AA medium) (Table 1) [63]. However, in some species, nutrient deficiency was not shown to be related to akinete formation. For example, *Anabaena cylindrica* did not form akinetes under nitrate-deficient conditions, but iron (Fe) deficiency did induce akinete formation (experimental condition: temperature 24.85 °C, light 22 μmol $m^{-2}s^{-1}$, BG 11 and MDN-N medium) (Figure 3. ④; Table 1) [61]. Moreover, *Anabaena crassa* did not form akinetes in the case of either phosphorus deficiency or nitrogen deficiency, but it did form heterocysts (experimental condition: temperature 20 °C, light 40 μmol $m^{-2}s^{-1}$, T medium) (Table 1) [34].

By contrast, *Anabaena circinalis* (Figure 3. ③; Table 1) [81] and *Cylindrospermopsis raciborskii* (Table 1) [62] required phosphorus for akinete formation. *A. circinalis* did not form akinetes under nitrogen (ammonium and nitrate) limitation, while akinetes were maximally

formed with the addition of 0.06 mg phosphorus (experimental condition: temperature 25 °C, light 30 μmol $m^{-2}s^{-1}$, N and P addition in ASM-1 medium) [81]. *C. raciborskii* formed akinetes at a very low density (10–20 akinetes mL^{-1}) under the addition of a phosphorus concentration of 0–3 μg L^{-1}, but the akinete density increased (1770–2310 akinetes mL^{-1}) with increasing phosphorus concentration (70–7000 μg L^{-1}), and the maximum akinete density appeared at a phosphorus concentration of 70 μg L^{-1} (experimental condition: temperature 25 °C, light 25 μmol $m^{-2}s^{-1}$, JM medium) [62]. These results suggest that some species of cyanobacteria require phosphorus at concentrations high enough to allow metabolic and physiological mechanisms to continue to ultimately lead to the successful formation of akinetes.

Cyanobacterial species such as *Dolichospermum circinale, Anabaena iyengarii*, *Nostochopsis lobatus*, and *Westiellopsis prolifica* have been shown to require both nitrogen and phosphorus for akinete formation. A combination of nutrient types (ammonium, nitrate, and phosphorus) was used by Park et al. [11]. In that experiment, *D. circinale* showed the maximum akinete density (420 akinetes g^{-1}) under conditions involving the addition of both ammonium and phosphorus (experimental condition: temperature 20 °C, light 30 μmol $m^{-2}s^{-1}$, -N-P, +N-P, -N+P, +N+P in CB medium) (Figure 3. ①; Table 1). Meanwhile, the density decreased to 140 akinetes g^{-1} under conditions involving the addition of nitrate and phosphorus. The effect of phosphorus alone, excluding nitrogen, was not found to be significant, but the highest number of akinetes was formed with the treatment of ammonium alone (540 akinetes g^{-1}). Notably, the effects of ammonium and nitrate on *D. circinale* akinete formation were different. Akinete density was high in all conditions where ammonium was added, while akinete density was significantly lower in conditions where nitrate was added. As a result, ammonium was found to be the most sensitive to *D. circinale* akinete formation compared to nitrate and phosphorus [11]. The three species of cyanobacteria *A. iyengarii*, *N. lobatus*, and *W. prolifica* also showed the highest akinete formation rates of 6%, 55%, and 70%, respectively, with the addition of both nitrogen and phosphorus (experimental condition: temperature 22 °C, light 40 μmol $m^{-2}s^{-1}$, -N-P, +N-P, -N+P, +N+P in Basal medium) [37]. However, *A. iyengarii* and *N. lobatus* showed no difference in the rate of dormant sporulation in the [+N+P] condition compared to the exclusion of phosphorus (+N-P) or nitrogen (-N+P), while *W. prolifica* showed a slight difference in this case (10–11%).

In relation to phosphorus, potassium ion (K^+) deficiency has also been shown to affect akinete formation. In Sukenik et al. [74], *A. crassa* formed akinetes in both types of medium with and without K^+, but the relative distribution of akinetes increased more than three-fold under K^+ deficient conditions, while the total number of akinetes per culture volume increased almost 40-fold (experimental conditions: temperature 20 °C, light 35 μmol $m^{-2}s^{-1}$, BG 11 medium). *Anabaena ovalisporum* formed maximum akinetes in K^+ deficient medium (0–0.47 mM); although K^+ deficiency also induced akinete formation, akinete accumulation decreased when phosphorus was removed from the medium [74].

Several previous studies have shown that nitrogen was a factor affecting akinete formation in cyanobacteria, but the effect was not constant, and it appeared to vary among species. *Nodularia spumigena* formed akinetes when nitrate was added under high light conditions [82], and *Anabaena torulosa* showed a doubled akinete formation rate when nitrate was added to N-free medium [83]. By contrast, *Anabaena doliolum* and *A. circinalis* showed increased akinete density in nitrate-free medium [76,80]. Meanwhile, an increase in *Nodularia spumigena* akinete density in Baltic Sea sediment was associated with a deficiency of dissolved inorganic nitrogen in the water [14]. However, *Anabaena muscosa*, *A. crass,* and *A. spiroides* did not form akinetes under nitrate-deficient conditions [34]. Moreover, nitrate did not affect akinete formation in *A. circinalis*, *Nostoc* PCC7524, or *Aphanizomenon flos-aquae* [26,81,84].

Under suitable temperature and light conditions, cyanobacteria growth and blooms have been shown to be regulated by nutrients [85]. Nitrogen is an essential nutrient for biological processes such as amino acid and protein synthesis [86]. Phosphorus determines

the survival of organisms through bioenergy synthesis [87]. Therefore, nitrogen and phosphorus limitation can negatively affect cyanobacteria growth and act as a potential trigger for akinete formation [43]. However, based on the results that have been presented in the literature, akinete formation may not only be the result of a stress response due to nutrient deprivation, but it may also be a metabolic response requiring nutrients. The mechanisms of akinete formation in cyanobacteria in the field with spatial and temporal variations in nutrient types and concentrations may be more complex, i.e., changes in the growth patterns and physiological activity of cyanobacteria may be involved in inducing akinete formation by interacting with a combination of environmental factors, including nutrients.

4. The Link between Akinete Formation and Germination in the Life Cycle of Cyanobacteria

In freshwater ecosystems, the life cycle of cyanobacteria of the Nostocales order occurs through akinetes and follows seasonal changes [14]. In spring, akinetes germinate in the sediment and are recruited into the water column, eventually leading to the development of vegetative cells [30,57,88]. During the summer, growth is stimulated by increased water temperature, and exponential growth develops into blooms [11,89]. The decrease in water temperatures from late autumn to winter leads to the occurrence of cell senescence and the formation of akinetes, which settle into the sediment. The akinetes overwinter in the sediment and germinate again the following spring, thus repeating the cycle of growth [56,90]. This year-round life cycle of akinete germination, development into vegetative cells, and subsequent akinete formation in temperate regions with large annual differences in water temperature demonstrates the connection between the water column (vegetative cell) and the sediment (akinetes) [56,90]. This seasonal variation in the life cycle of cyanobacteria also allows us to recognize akinete formation and germination as a temporally (winter vs. spring) and spatially (water column vs. sediment) separated phenomena.

However, on a finer scale, it is likely that akinete formation and germination in the field may be more strongly linked than we currently recognize, at least during certain times of the year. As discussed above, the range of environmental factors that initiate or trigger akinete germination in various species of cyanobacteria is broadly consistent with those that promote vegetative cell growth [11,19,27]. These results suggest that akinete germination can occur year-round in the field as long as the water temperature at which germination is possible is within the range of 10–35 °C [19]. It also appears that unfavorable conditions for growth could induce a vegetative cell to form akinetes [33,63]. However, vegetative cells also form akinetes during active growth under suitable environmental conditions [11,32,72,78]. This raises the question of the role of akinetes produced in large quantities in the water column during the exponential growth period.

Anabaena flos-aquae formed akinetes in two stages in the Bugach reservoir in Russia during a high-temperature summer bloom [32]. In the first phase, which occurred from June to July, akinetes were mostly distributed in the water column (within vegetative cell filaments), and the density of akinetes in the sediment did not increase during this period. This was evidenced by the fact that most of the akinetes were not shown to settle into the sediment but instead germinated in the water column. The second phase occurred from August to September when the vegetative cell densities were lower or similar to the first phase, but the akinete densities were higher. During this period, the akinete density in the sediment increased rapidly. Park et al. [11] also observed that, in a growth chamber composed of the water column and the sediment part in a cylinder, more than 60% of *Dolichospurmum circinale* akinetes that had formed in the water column during the exponential growth period were converted to empty shells (post-germinated shells) at the bottom of the chamber. This indicated that formation and germination occurred together during the experimental period. From these results, it can be understood that the akinetes produced during vegetative cell development perform two functions: maintaining the population in the water column through vegetative reproduction (germination)

and transitioning to a resting stage for overwintering. High water temperatures during the summer constitute favorable conditions for triggering akinete germination and enable active vegetative cell growth. Compared to recruitment into the water column via germination from the sediment, direct germination in the water column would be more advantageous for maintaining populations at high densities in the water column during the exponential growth period. On the other hand, akinetes that have been deposited in shallow sediment environments provided with adequate light and temperature might have the potential to germinate readily into vegetative cells [45,57]. Shallow sediment areas, such as the littoral zone, can be hot spots for akinetes as well as other harmful cyanobacteria (e.g., *Microcystis)* to germinate or become active, enter the water column and develop into blooms [56,91]. From an annual life cycle perspective, this indicates that akinete formation and germination are seasonally decoupled phenomena but that they may be synchronized during exponential growth periods such as summer. Moreover, if these akinetes are present in shallow sediments with suitable environmental conditions for germination, these two phenomena may become closely linked spatially (benthic–pelagic coupling), even during the same season.

Based on the results of akinete formation and germination discussed above, this review altogether highlights that the life cycle of cyanobacteria from the Nostocales order following the annual cycle can be highly dynamic in situ, both temporally and spatially. We propose a revised conceptual model of the life cycle of akinete-forming cyanobacteria (e.g., *D. circinale*) illustrated in Figure 5, underlining the tight coupling of akinete formation–germination. It is possible for short-term cycling (formation–germination–growth) to occur in the water column, particularly during the summer bloom, and in shallow areas, there may be strong spatial connectivity between the sediment and water column, which allows for recruitment into the water column via germination and growth to occur. The stronger the connectivity between the water column and the sediment, the more likely it is that harmful algal blooms will occur throughout the year or for multiple years; moreover, various factors affect the degree of connectivity, including the extent of akinete accumulation in the sediment, water depth, soil texture, and sediment disturbances.

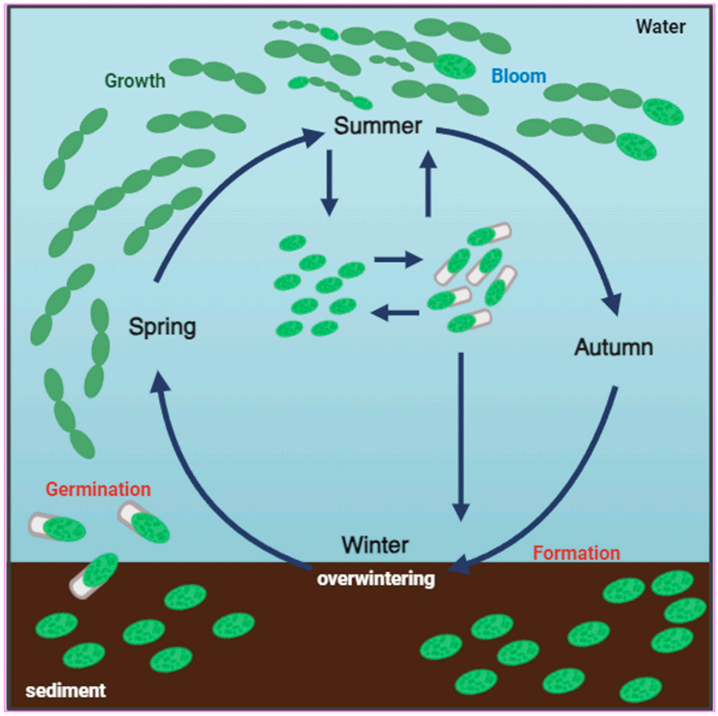

Figure 5. Conceptual model of the proposed life cycle of *Dolichospurmum circinale* (Nostocales order) highlighting the role of akinetes in the summer season (coupling of formation and germination).

5. Conclusions

This review examined the environmental tolerances (mainly temperature, light intensity, and nutrients) to the germination and formation of akinetes, which constitute the life cycle of the Nostocales cyanobacteria and are used in their survival strategies. We identified that the environmental tolerances to germination, formation, and vegetative cell growth vary among different species and strains of cyanobacteria, but the ranges are often overlapping. The range of environmental factors that induce akinete formation and germination has largely been determined in laboratory settings, and environmental factors have almost always been assessed in isolation, albeit at a range or nominal concentration, and this may deviate from the true tolerance ranges in the field, where multiple factors are always present simultaneously. This is likely due to a combination of factors, including differences in the environmental conditions under which akinetes are harbored. Consequently, in sites featuring multiple and complex environmental factors, it is not likely that any single physical, chemical, or biological factor plays a decisive role in inducing akinete formation and germination, but it is instead likely that multiple factors act through changes in the growth pattern and physiological activity of the cyanobacteria. By considering the results of many prior studies in this area, the present review identifies the possibility that akinete formation and germination are not simply separated seasonally within the annual life cycle of cyanobacteria but that they may occur simultaneously within a short period of time depending on the season, and this review suggests the potential existence of a link between akinete formation and germination in the water column (formation–germination–growth) and a link between the sediment and the water column during large blooms, such as in summer. This highlights the need to further elucidate the annual life cycle of akinete-forming cyanobacteria. Such information will not only help improve our shared understanding of the life cycle of cyanobacteria, but it will also provide insights into the monitoring and management of harmful cyanobacterial blooms. This information is also expected to help guide the selection of sites and effective treatments for harmful algal bloom management, which can be important for proactively identifying a harmful algal bloom in the field and developing countermeasures. Connection of the sediment and water column through akinete is ecologically important as an adaptive strategy for the survival of cyanobacteria. Therefore, to better understand the mechanisms of akinete development, it is important to study the life cycle of cyanobacteria, which includes not only the succession of life (birth, growth, and death) but also the geomorphological and environmental characteristics of areas where cyanobacteria occur. This review does not address the regulation of akinete formation and germination, i.e., the molecular mechanisms of akinete germination, formation, and maturation. In recent years, the application of transcriptomic, proteomic, and metabolomic tools to akinetes has yielded mixed results ([89] and references therein), and these areas still remain largely unexplored. There is a need for such regulatory studies to continue being conducted in the future to gain a more fundamental understanding of the cyanobacterial akinete life cycle.

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