

Review

Drought adaptation of a terrestrial macroscopic cyanobacterium, *Nostoc flagelliforme*, in arid areas: A review

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***Nostoc flagelliforme* is a terrestrial macroscopic cyanobacterium with a hair-like appearance that is distributed in arid and semi-arid areas. It exhibits remarkable tolerance to extremely acute or long-term dehydration stress. However, it cannot withstand prolonged hydration or water saturation. It appears that too little water limits its growth while too much water becomes a burden. This seemingly contradictory situation is particularly pronounced in *N. flagelliforme*, reflecting its distinctiveness among *Nostoc* species with respect to the ways they adapt to dry environments. In this review, we call attention to potentially important drought-adaptation mechanisms in this species, including mechanisms related to its morphology, structure, ecophysiology and genetic background, summarizing the available studies relevant to this topic. This species may become a good model for investigating the diverse genetic mechanisms of *Nostoc* adaptation to dry environments.**

Key words: Cyanobacteria, drought adaptation, dry environment, genetic mechanism, hydration cycle, *Nostoc flagelliforme*.

INTRODUCTION

Nostoc flagelliforme is a terrestrial macroscopic species with a hair-like appearance that is distributed in arid and semi-arid areas in many countries (Qian et al., 1989; Gao, 1998). It has been considered a delicacy in China for more than 2,000 years and is called "Facai" in Chinese, which means "getting rich" (Takenaka et al., 1998). Chinese scientists have focused on exploring the ability of *N. flagelliforme* to form crusts for controlling desertification (Chen et al., 2011) and artificially cultivating it for use in the food and medical industries (Liang et al., 2007; Yu et al., 2010). In recent years, frequent droughts have been a prominent global problem, leading to deterioration of the environment and severe reductions in crop production. Considering these circumstances, understanding the molecular and genetic mechanisms by which *N. flagelliforme* adapts to extreme dry environments is of great significance (Wu et al., 2011;

Liang et al., 2011; Liu et al., 2012). Studies on this topic have the potential to provide valuable information for developing transgenic drought-tolerant crops to among others cope with the current food crisis.

The native habitats of *N. flagelliforme* are highly varied, being characterized by high evaporation rates, substantial temperature differences, intense solar radiation and poor nutrient availability (Qian et al., 1989; Gao, 1998). Extreme dryness is the primary feature of the environment of this species, and water availability is the key limiting factor for its growth (Tang et al., 2005; Ye et al., 2006). Laboratory and field studies have shown that *N. flagelliforme* cannot survive continuous hydration for a certain time, despite the fact that sufficient water supports the growth of this species (Scherer and Zhong, 1991; Liang et al., 2007). This characteristic feature of *N. flagelliforme* distinguishes it from other terrestrial *Nostoc* species, such as its closest relative *Nostoc commune* (Scherer and Zhong, 1991; Li and Liu, 2003), and reflects the diversity of ways in which cyanobacteria have adapted to dry environments. In the past 30 years, *N.*

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flagelliforme has attracted increasing attention from Chinese scientists, and many of the studies on this species have been published in Chinese. The current review describes the multiple drought adaptation mechanisms of this species by summarizing the available relevant studies, with the aim to promote research on its molecular adaptive mechanisms. Also, *N. flagelliforme* has been compared with its close relative *N. commune* in some respects (Table 1), in order to help explain its typical characteristics.

CONDUCTIVENESS OF *N. FLAGELLIFORME* MORPHOLOGY IN COPING WITH DRY ENVIRONMENTS

In arid deserts and steppes, small shrubs often exhibit needle-like or waxy leaves, which are less prone to desiccation than wider, flatter-shaped leaves. Somewhat similarly, the overall shape of *N. flagelliforme* is cylindrical or hair-like, consisting of numerous rosary-like cells arranged in parallel and encased in a thick sheath (Chen et al., 2011). This hair-like filament is usually 5 to 60 cm long, 0.2 to 1 mm in diameter, and exists in nature as a curved mass on the soil surface. Under the microscope, the surface of the filament is rough, with multiple irregular colloidal blocks, finger-like protrusions and cracks (Tang et al., 2005). This rough surface may be beneficial for gas exchange, heat release and moisture absorption (Tang et al., 2005).

The cylindrical shape of *N. flagelliforme* results in a higher surface to mass (S/M) ratio compared to organisms with a lamellate shape. Scherer et al. (1984) reported that the S/M ratio of *N. flagelliforme* was approximately $82 \text{ cm}^2 \text{ g}^{-1}$, which is far greater than that of *N. commune* ($\sim 37 \text{ cm}^2 \text{ g}^{-1}$), meaning that *N. flagelliforme* is able to take up water from the surrounding environment more quickly. Rapid water uptake was found to be completed after 10 min (Scherer et al., 1984), and the weight of air-dried *N. flagelliforme* increased to up to 7 times its original weight in 2 min when it was rehydrated (Liu et al., 2010). The half-times of rewetting are 0.6 and 3.3 min for *N. flagelliforme* and *N. commune*, respectively, inversely proportional to their S/M ratios (Scherer et al., 1984). The cylindrical shape of *N. flagelliforme* is therefore advantageous for uptake of water as intermittent rainfall or dew that forms at night and in the early morning for its survival in arid areas (Scherer and Zhong, 1991).

A higher S/M ratio is also correlated with a higher rate of water loss upon high temperatures and windy conditions. *N. flagelliforme* loses water more quickly than *N. commune* (Scherer et al., 1984). It takes approximately 6 h for rehydrated *N. flagelliforme* to air dry (Scherer et al., 1984; Tang et al., 2005). During this short drying period, rehydrating *N. flagelliforme* accumulate dry matter (Gao, 1998; Bi and Hu, 2003), a process that is

enhanced by moderately dry conditions (Zhao et al., 2000). Theoretically, an increased water-retention time is expected to allow colonies to accumulate more dry matter. Thus, the slow growth of *N. flagelliforme* may be partially attributable to its short water-retention period. In contrast, *N. commune* grows faster, and rehydrated *N. commune* desiccate in approximately 12 h, which is nearly twice the water retention time of *N. flagelliforme* (Scherer et al., 1984). However, it is unfavorable for *N. flagelliforme* to remain hydrated for a prolonged period of time, as this leads to a significant decline in nitrogenase activity and loss of sheath integrity (Wang and Zhang, 1988; Bi and Hu, 2003). The reasons for this phenomenon will be discussed as the study proceeds. In addition, rapid water loss is important for *N. flagelliforme* to avoid heat damage after rain in the summer, when the surface temperature of the soil can rise to 66°C (Qian et al., 1989; Gao, 1998). Therefore, rapid water loss is physiologically and ecologically necessary for *N. flagelliforme* to survive in dry environments.

Lamellate *N. flagelliforme* can be found sporadically distributed in microhabitats with an adequate water supply, but they are rarely found in extremely arid regions (Tang et al., 2003). When cylindrical *N. flagelliforme* samples were cultivated in the field and intermittently supplied with adequate water for 4 months, nearly all of them were observed to grow into the lamellate shape (Tang et al., 2000). The presence of water seems to be the main environmental factor affecting *N. flagelliforme* morphogenesis. The lamellate shape of *N. commune* may account for its scarcity in the arid regions where *N. flagelliforme* thrives. For example, in Siziwangqi county in Gansu province, China, *N. commune* and *N. flagelliforme* co-exist in regions with annual precipitation of approximately 300 mm per year, while the abundance of *N. flagelliforme* increases from south to north associated with decreasing annual precipitation, which can be as low as 100 to 140 mm per year in the northernmost regions (Scherer and Zhong, 1991; Li and Liu, 2003). Thus, the predominant hair-like shape of *N. flagelliforme* is an important strategy for helping it cope with dry environments.

THE CRUCIAL ROLE OF EXTRACELLULAR POLYSACCHARIDE SHEATH FOR PROTECTION AGAINST DESICCATION STRESS

Production of extracellular polysaccharides (EPS) is widespread among cyanobacteria. *N. flagelliforme* EPS is composed of four neutral sugars, glucose (43.2%), xylose (20.6%), galactose (29.9%), and mannose (6.3%) (Jia et al., 2007). Also, the polysaccharides are non-sulfated, contain β -glycosides, present a decomposition peak at 245°C (Yu and Jia, 2008) and can be secreted into liquid media or soil (Hua et al., 2003; Yu et al., 2010). Research addressing *N. flagelliforme* EPS has thus far focused on

Table 1. Differences between *N. flagelliforme* and *N. commune* with respect to drought tolerance.

S/N	Species	<i>N. flagelliforme</i>	<i>N. commune</i>
1	Climate*	Arid and semiarid climates (Scherer and Zhong, 1991)	Semi-arid and temperate climates (Scherer and Zhong, 1991)
2	Location*	Mainly 1000 - 2800 m above sea level (Gao, 1998)	Mainly 450 - 900 m above sea level (Li and Liu, 2003)
3	Annual precipitation*	< 400 mm; generally < 200 mm (Tang et al., 2000)	generally > 300 mm (Scherer and Zhong, 1991)
4	Main shape	Mostly cylindrical; fewer lamellate (Gao, 1998)	Lamellate (Li and Liu, 2003)
5	Water uptake and loss of the colonies	Half-time of rewetting, 0.6 min; desiccation time in air, 350 min (Scherer et al., 1984)	Half-time of rewetting, 3.3 min; desiccation time in air, 700 min (Scherer et al., 1984)
6	Survival on wet filter paper	After 4 to 5 days, colonies disintegrated (Scherer and Zhong, 1991)	After 4 to 5 days, cells survived and formed daughter colonies (Scherer and Zhong, 1991)
7	ROS scavenging effects of EPS	Hydroxyl radicals (maximum 74.3%) and superoxide radicals (maximum 35.5%) (Tang and Hu, 2006)	Hydroxyl radicals (maximum 46.7%) and superoxide radicals (maximum 72.3%) (Tang and Hu, 2006)
8	Unsaturated fatty acids in membrane lipids	(MUFAs + PUFAs) / total, 72.5% for flask culture and 76% for natural colonies (Liu et al., 2005)	(MUFAs + PUFAs) / total, 70% (Liu et al., 2003)

*, In China.

examining various culture conditions to achieve high EPS production for possible commercial use (Yu and Jia, 2008; Yu et al., 2010). Therefore, our understanding of the importance of EPS in desiccation tolerance comes mainly from studies of *N. commune*, which is a useful model for studying the desiccation tolerance of prokaryotic cells (Potts, 1994; Tamaru et al., 2005).

In addition to determining the shape of its colonies, the sheath of *N. flagelliforme* is pivotal in protecting cells from desiccation stress. During the developmental stage, when the sheath is not formed, cells are susceptible to environmental changes, and a thicker sheath appears to increase the probability of survival (Gao, 1998). In EPS-depleted *N. commune* cells, photosynthetic O₂ evolution was significantly damaged by desiccation, and the viability of these cells decreased after air drying (Tamaru et al., 2005). Multiple roles have been suggested for EPS in stress tolerance. EPS can inhibit the fusion of membrane vesicles during desiccation and freeze-drying and are, thus, important for maintaining the structure and function of biological membranes during desiccation (Hill et al., 1997). EPS provides a flexible structural scaffold to accommodate the rapid biophysical and physiological changes that occur in response to changing environmental conditions (Helm et al., 2000). EPS can also act as an immobilization matrix for accumulating secreted proteins, such as superoxide dismutases

(SOD), to counteract long-term desiccation stress (Shirkey et al., 2000; Dadheech, 2010). In addition, EPS may serve as a sink for excess fixed carbon (or energy) when C/N metabolism is unbalanced (Tamaru et al., 2005). It was estimated that adenosine triphosphate (ATP) synthesis is similar in *N. flagelliforme* and *N. commune* (112 and 124 μmol/g dry weight/h, respectively), but the total ATP consumption required for N₂ and CO₂ fixation in *N. flagelliforme* (24-32) is much lower than in *N. commune* (199 to 263) (Scherer and Zhong, 1991). It is reasonable to speculate that the energy saved via slower growth also enhances the ability of *N. flagelliforme* to survive in adverse environments.

An important role for the polysaccharide sheath of *N. flagelliforme* is to provide an extracellular defense mechanism against attack by reactive oxygen species (ROS). Natural *N. flagelliforme* habitats are usually 1,000 to 2,800 m above sea level and exposed to strong ultraviolet radiation (Gao and Ye, 2007). Ultraviolet radiation and water stress increase the generation of ROS, which results in lipid peroxidation, denaturation of proteins and nucleic acid damage (França et al., 2007). It has been reported that EPS extracted from *N. flagelliforme* can scavenge superoxide anions and hydroxyl radicals, with efficiencies of 35.5 and 74.3% respectively (Tang and Hu, 2006). EPS from *N. commune* are also capable of scavenging both types of radicals but,

are more effective in scavenging superoxide radicals (Tang and Hu, 2006; Li et al., 2010). While this indicates that there may be differences in the chemical properties of EPS between these two closely related species, determining the ROS-scavenging efficiency of EPS *in situ* requires further investigation. Unsaturated fatty acids occupy 72.6% of the total fatty acids in *N. flagelliforme* membrane lipids, which is the highest percentage reported thus far in cyanobacteria (Wang et al., 2000; Liu et al., 2003), and these molecules are highly susceptible to oxidative attack (Singh et al., 2002). Therefore, the prevention of lipid peroxidation by EPS is critical for *N. flagelliforme* cell survival when it is subjected to ultraviolet radiation or desiccation stress.

In addition to the colony morphology, the physicochemical properties of *N. flagelliforme* EPS is responsible for its efficient water absorption. Colony morphology primarily functions in determining the velocity of water uptake. Field observations showed that 50% air humidity or 30% soil moisture can sustain *N. flagelliforme* growth (Zhang and Zhang, 2003). In the native habitats of this species, the relative humidity is below 60% during daytime and up to 80 to 90% at night, and water vapor turns into dew when the temperature drops at night (Gao, 1998). Dew and occasional rain are therefore the main sources of water for *N. flagelliforme* (Tang et al., 2005). Therefore, when rain water is not available, EPS plays a critical role in absorbing dew or moisture from the air for growth of *N. flagelliforme*. It is also likely that the slow growth, a result of the limited availability of water from dew or moist air, maintains the essential repair activity of the cells, especially during the long rainless season.

THE CRUCIAL STATE OF PHYSIOLOGICAL ADAPTATION TO THE HYDRATION CYCLE IN THE DRY ENVIRONMENT

N. flagelliforme is dormant most of the time (Zhao et al., 2008). In its native habitats in China, precipitation occurs mainly from June to September (Ye et al., 2008). Brief or moderate rainfall and subsequent high temperatures often lead to swelling and shrinkage of the filaments of *N. flagelliforme* (Tang et al., 2005). Therefore, *N. flagelliforme* is constantly undergoing either short- or long-term cycles of rehydration and dehydration in dry environments, as shown in Figure 1. However, *N. flagelliforme* cannot endure prolonged hydration or water saturation, as noted earlier. For example, after 4 to 5 days on fully hydrated filter paper, colonies of *N. flagelliforme* disintegrated, and no growth was detected, whereas *N. commune* not only survived but formed daughter colonies (Scherer and Zhong, 1991). Under field cultivation, frequent watering leads to death of *N. flagelliforme* (Liang et al., 2007). Thus, too little water limits growth, while too much water becomes a burden. This seemingly contradictory situation is particularly

pronounced in *N. flagelliforme*, reflecting its distinctiveness among *Nostoc* species with respect to the ways they adapt to dry environments.

Epiphytic bacteria have been recognized as the main reason for the disintegration of *N. flagelliforme* colonies. Indeed, an investigation of the ultra-structure of vegetative cells revealed a large number of bacteria in *N. flagelliforme* sheaths (Zhu et al., 1998). Moreover, when bacteria were inhibited in aquatic culture, *N. flagelliforme* filaments did not disintegrate and instead maintained sheath integrity for approximately 20 days (Gao and Ye, 2003). In the soil of the fixed and semi-fixed deserts where *N. flagelliforme* thrives, there are fungi and aerobic bacteria including actinomycetes (Zhang and Zhang, 2003). This is reasonable because *N. flagelliforme* is an important primary producer in bare and arid areas, where its EPS serve as a valuable source of organic carbon for microorganisms. Bacteria (identified as *Bacillus* by 16S rRNA sequence analysis) have been isolated from air-dried *N. flagelliforme* that were stored for 3 years (Gao et al., 2011). Therefore, it is reasonable to hypothesize that prolonged hydration leads to excessive proliferation of bacteria and results in the disintegration of the sheath (Gao, 1998; Gao and Ye, 2003; Liang et al., 2007). Dry *N. flagelliforme* can tolerate extreme high-temperature stress, but rehydrated *N. flagelliforme* disintegrate readily under this condition (Tang et al., 2000; Liu et al., 2005), which may be explained by this hypothesis.

Epiphytic bacteria may exert long-term selective pressure on *N. flagelliforme* survival, which is reflected in its microhabitats. The various types of soil that support *N. flagelliforme* growth share a few common characteristics: a solid surface with a certain degree of compaction; thin, cracked and forming crust; and underneath a large amount of loose sand (Zhao et al., 2000). This special structure of the soil guarantees that the entire surface of *N. flagelliforme* is in full contact with the soil, which is advantageous for absorbing adequate amounts of water when it rains; at the same time, it can avoid gathering too much water on the soil surface (Zhao et al., 2000). In addition, *N. flagelliforme* is usually found on slopes or in raised areas, but it is not found in low-lying areas that easily accumulate water; moreover, there are more colonies of this species found growing in windward slopes than leeward slopes (Zhao et al., 2000). Of course, strong and frequent wind is extremely detrimental to its growth (Zhang and Zhang, 2003). Taking these findings together, a combination of environmental abiotic (water) and biotic (bacteria) factors drives *N. flagelliforme* to survive in specific microhabitats.

The effects of bacteria on the growth of *N. flagelliforme* have not been well studied. The complete picture of the relationship between them may not be limited to the two well-established facts, which are as follows: 1) excessively proliferating bacteria are inhibitory or lethal to *N. flagelliforme*; and 2) the organic matter produced by *N. flagelliforme* nourishes soil microorganisms, and these

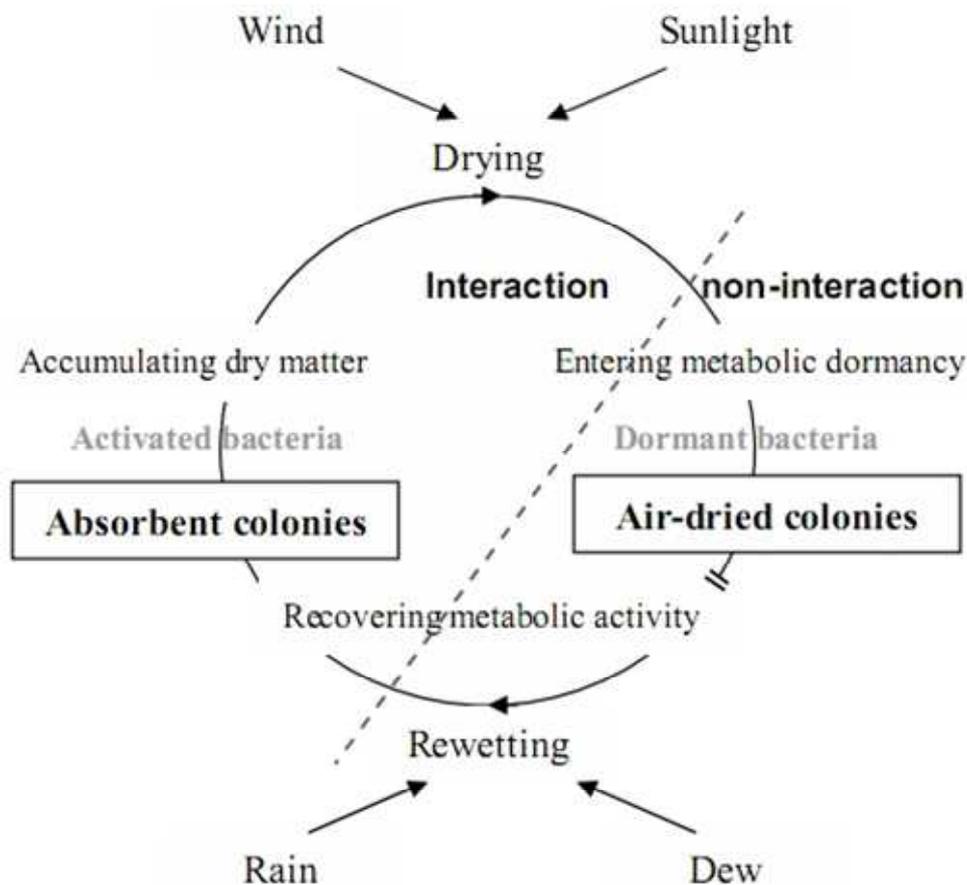


Figure 1. A model for the potentially favorable interaction between *N. flagelliforme* and epiphytic bacteria during repeated cycles of dehydration and rewetting in arid areas.

microorganisms, in turn, metabolize soil minerals that promote *N. flagelliforme* growth (Zhang and Zhang, 2003). In the practice of artificial cultivation of *N. flagelliforme*, Qian et al. (1989) first raised the "hydration cycle" concept, that is, it is critical to mimic the natural ecology of this species by keeping the material under alternative "dry-wet-dry" moisture cycles. This technique has proven to be successful in the field cultivation of *N. flagelliforme* (Li and Hu, 2003; Liang et al., 2007). Periodic desiccation inhibits the excessive proliferation of bacteria and, thus, protects the sheath from bacterial decomposition (Gao and Ye, 2003). More importantly, this shift in dry/wet status promotes nitrogenase activity and the accumulation of dry matter and can also increase resistance to drought stress (Zhong et al., 1992). Otherwise, there is no net accumulation of photosynthetic carbon and instead *N. flagelliforme* consumes the original dry matter due to the increased respiration, when it is soaked in water (Liang et al., 2007). Therefore, the *N. flagelliforme* "hydration cycle" seems to have evolved as an adaptive characteristic for surviving in dry environments. Epiphytic bacteria presumably played a

role in promoting this evolutionary process due to long-term mutual interactions, as hypothesized in Figure 1. In this model, *N. flagelliforme* colonies and epiphytic bacteria undergo short-term rehydration and relatively long-term drying processes together interspersed with varied periods of desiccation. Upon rewetting by rain or dew, the shrunken sheath begins swelling, during which time the colonies rapidly recover their metabolic activity, and the bacteria become activated. The activated bacteria digest polysaccharides by secreting enzymes, which helps loosen the rigid polysaccharide sheath and, thus, relieves pressure on the expanding cells; at the same time, the metabolites secreted by the bacteria potentially stimulate the rapid growth of colonies. When the temperature rises after a rainfall event, rewetted colonies experience rapid water loss, during which both the colonies and bacteria gradually reenter dormancy, effectively avoiding adverse effects brought about by excessive bacterial proliferation. Subsequently, they will endure either long or short periods of desiccation together. A recent observation has suggested this possibility. We observed that in the aquatic culture of

N. flagelliforme sample without any sterile processing (40 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and 25°C), photosynthetic activity (in terms of Fv/Fm) did not decrease after 24 h, when sheath integrity had been lost due to the large number of bacteria present; instead, photosynthetic activity reached its highest value around that time. This phenomenon suggests that epiphytic bacteria may not be as harmful to *N. flagelliforme* cells as previously thought although it can digest the sheath of *N. flagelliforme*.

ESSENTIALITY OF THE GENETIC BACKGROUND FOR REMARKABLE DROUGHT TOLERANCE

The molecular genetic mechanism underlying drought adaptation in *N. flagelliforme* is not well understood. However, it is encouraging that there is increasing attention paid to it (Wu et al., 2010, 2011; Liang et al., 2011; Gao et al., 2011; Liu et al., 2012). BLAST analyses of the partial genomic sequence and putative proteins indicated that *N. flagelliforme* is most closely related to *Nostoc punctiforme* PCC 73102 among the completed genomes of cyanobacterial species (Gao et al., 2011). There are over 400 genes potentially related to sensing and responding to environmental signals in the *N. punctiforme* genome (Meeks et al., 2001). Also, *N. punctiforme* has no thick gelatinous sheath and is found predominately in terrestrial habitats in symbiosis with fungi, bryophytes, gymnosperms and angiosperms (Meeks et al., 2001). Thus, we speculate that within the evolutionary lineage of *N. flagelliforme* and its closely related species, the formation of a thick sheath may have played a decisive role in evolution from a symbiotic ecotype (*N. punctiforme*) to an ecotype that can grow on the soil surface (*N. flagelliforme*). Recently, some osmotic stress up-regulated cDNA fragments have been isolated in *N. flagelliforme* and the proteins represented by them, such as ATPases, methyltransferases, transporters, glutathione synthase and signal transduction-associated components possibly play an important role in *N. flagelliforme* coping with the early phase of drought stress (Liu et al., 2012). Many transposases are also predicted to exist in the *N. flagelliforme* genome, which would offer a great evolutionary flexibility to help it cope with the dry environments (Gao et al., 2011). In addition, some biochemical and physiological adaptations have also occurred in *N. flagelliforme*. For example, monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) comprise 38.5 and 34.1%, respectively, of the total fatty acids in the membrane lipids of *N. flagelliforme*, representing the highest percentages reported so far among all cyanobacteria (Wang et al., 2000; Liu et al., 2003). Increased proportion of unsaturated fatty acids enhances tolerance to water, salt, strong light and low temperature stresses (Singh et al., 2002). The activities of extracellular and intracellular carbonic anhydrases (CA)

are differentially regulated by cycles of hydration and dehydration, which is associated with the changing pH of the microhabitats (Ye et al., 2008). Taken together, these findings indicate that *N. flagelliforme* has evolved extensive genetic mechanisms for coping with dry environments.

Other biochemical and physiological mechanisms have also been reported recently in *N. flagelliforme* that enable it to cope with oxidative, osmotic and ionic stresses. Fe-SOD remained active in *N. flagelliforme*, even after 3 years of dry storage, and its activity was 78% of that in the fully recovered samples (Qiu et al., 2003). In comparison, in *N. commune* CHEN/1986, active Fe-SOD was the third most abundant soluble protein in cells that had been desiccated for 13 years (Shirkey et al., 2000). The high stability of Fe-SOD should be critical for *N. flagelliforme* to cope with oxidative stress. Recently, *N. flagelliforme* peroxiredoxin has been isolated and it may play an important role in regulating the intracellular hydrogen peroxide level during the cycles of dehydration and rehydration (Liang et al., 2011). The water potential of air-dried *N. flagelliforme* is -8.2 MPa, which is far lower than that observed in desert plants such as *Artemisia*, *Salix* and *Xinjiang calligonum* (Tang et al., 2005). Potentially, trehalose plays an important role in protecting *N. flagelliforme* cells against the damage of osmotic stress. Maltooligosyltrehalose trehalohydrolase (MTH) and maltooligosyltrehalose synthase (MTS), two enzymes that are responsible for trehalose synthesis, were significantly up-regulated in *N. flagelliforme* under osmotic, salt and high-temperature stresses (Wu et al., 2011); Trehalose and sucrose concentrations increase rapidly in the early stage of drought stress and subsequently maintain a high level (Pang et al., 2007). Similar research also suggested that trehalose is important for dehydration tolerance in *Anabaena* sp. PCC 7120 (Higo et al., 2006). In addition, *N. flagelliforme* cells should extrude sodium ions and accumulate potassium ions to maintain internal ionic balance upon dehydration stress, as reported in other cyanobacteria (Billi and Potts, 2002; Dadheech, 2010), as 0.1 mM NaCl does not obviously affect its photosynthetic rate or chlorophyll fluorescence intensity (Zhao et al., 2005) and the addition of exogenous potassium enhances its photosynthetic recovery upon rehydration (Qiu et al., 2004). These findings indicate that *N. flagelliforme* cells share some universal drought-adaptation mechanisms as reported for other cyanobacteria (Potts, 1999; Apte, 2001; Dadheech, 2010).

CONCLUSION

The abilities of *Nostoc* strains to withstand short- or long-term desiccation and to recover metabolic activities fairly soon after rewetting are crucial for their success in dry terrestrial environments. However, the diversity of

environmental ecologies inhabited by these cyanobacteria has also conferred some distinctive adaptation mechanisms upon them. *N. flagelliforme* is unique with respect to the disintegration of its sheath under conditions of prolonged hydration or water saturation. Epiphytic bacteria may exert strong selective pressure on the evolution of this mechanism. Severe disintegration of the sheath will eventually lead to death of *N. flagelliforme*, but it rarely takes place in its natural habitats. The hair-like morphology of *N. flagelliforme*, the biochemical properties of its EPS and the specific microhabitats it populates, which promote rapid water uptake and loss, may account for this good adaptation. Therefore, we hypothesize that the "hydration cycle" physiology has evolved in *N. flagelliforme* in response to the long-term combined pressures of water stress and epiphytic bacteria in dry environments.

The remarkable resistance of *N. flagelliforme* to desiccation stress is essential for its survival in arid areas. *N. flagelliforme* has evolved extensive genetic mechanisms to adapt to drought conditions, such as the possibility that many transposases exist in its genome, forming thick gelatinous sheath, etc. Cross-adaptation mechanisms also exist between extreme drought and other environmental stresses, such as high temperatures, ultraviolet irradiation, or freezing. A good example is that the high proportion of unsaturated fatty acids in its membrane lipids is advantageous for membrane phase transition under various stresses. In future research, obtaining the full genome sequence of *N. flagelliforme* and establishing tools for its genetic manipulation will be critical for exploring the concrete molecular mechanisms of such specific and remarkable drought adaptations. This species may become a good model for investigating the diverse genetic mechanisms of *Nostoc* adaptation to dry environments.

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