RESEARCH ARTICLE

Estimation of growth and exopolysaccharide production by two soil cyanobacteria, *Scytonema tolypothrichoides* and *Tolypothrix bouteillei* as determined by cultivation in irradiance and temperature crossed gradients

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Two filamentous cyanobacteria of the genera *Scytonema* and *Tolypothrix* were reported to be effective for stabilizing soil in arid areas due to the production of significant amounts of extracellular polysaccharides (EPS). These EPS may also have applications in the biotechnology industry. Therefore, two cyanobacterial species, *Scytonema tolypothrichoides* and *Tolypothrix bouteillei* were examined using crossed gradients of temperature (8–40°C) and irradiance (3–21 W m⁻²) to identify their temperature and irradiance optima for maximum biomass and EPS production. According to their reported temperature requirements, both strains were considered mesophilic. The optimum growth range of temperature in *S. tolypothrichoides* (27 to 34°C) was higher than *T. bouteillei* (22–32°C). The optimum irradiance range for growth of *S. tolypothrichoides* (9–13 W m⁻²) was slightly lower than *T. bouteillei* (7–18 W m⁻²). Maximum EPS production by *S. tolypothrichoides* occurred at similar temperatures (28–34°C) as *T. bouteillei* (27–34°C), both slightly higher than for maximum growth. The optimum irradiance range for EPS production was comparable to that for growth in *S. tolypothrichoides* (8–13 W m⁻²), and slightly lower in *T. bouteillei* (7–17 W m⁻²). The Redundancy Analysis confirmed that temperature was the most important controlling factor and protocols for field applications or for mass cultivation can now be developed.

KEYWORDS

biological soil crusts, cyanobacteria, extracellular polysaccharides (EPS), irradiance, temperature

1 | INTRODUCTION

Production of an extracellular matrix in the form of various types of mucilaginous envelopes, capsules, or sheaths represents one of the most successful strategies for stress avoidance and/or stress tolerance in cyanobacteria [1]. Extracellular

polysaccharides (EPS) play a crucial role in desiccation tolerance as these molecules bind water [2,3]. Colonies of *Nostoc* sp. were capable of photosynthesis even when they had lost 80% of their weight and were in a dehydrated state [4]. The EPS also provide a matrix for extracellular sunscreen pigments, such as scytonemin, that protects the cells from UV radiation [5]. Accumulation of EPS is also used as a Carbon sink (C sink) during unbalanced growth [6].

In addition to providing protection for cells, EPS significantly contribute to stabilization of soil surfaces in arid

Abbreviations: ANOVA, analysis of variance; DW, dry weight; EPS, extracellular polysaccharides; GAM, generalized additive models

areas [7,8]. If small soil particles are bound to EPS and sheaths, colonization of surface layers of disturbed soils by cyanobacteria leads to the development of biological soil crusts and decreases the rate of soil erosion [9]. Filamentous cyanobacteria species of the genera *Scytonema* and *Tolypothrix*, with a structured sheath layer around their trichomes, have been reported to be effective in soil stabilization [7,8]. The ability of EPS to store water also improves the water-holding capacity of soils [8]. In arid soils, cyanobacterial growth adds considerable amounts of C and fixed N into the soil ecosystem [10]. Therefore, knowledge of their requirements for growth and EPS production would help in the development of a protocol for inoculum preparation and timing of its application in the field, resulting in more efficient soil stabilization and improvement of soil quality in arid areas.

Biotechnological applications of cyanobacterial EPS are also promising [11,12]. They have been used as a bioflocculant in water treatment management and heavy metal removal [13–15]. Due to the ease of cultivation, they are also used for production of biopolymers for various applications [16]. Their antiviral and antitumor activities are also used in drug discovery and development [17,18]. The biological soil crust can be considered as a form of biofilm (see definition of biofilm by Kvíderová [19]), and the majority of applications of the biofilm were summarized by Roeselers et al. [20]; the EPS of a cyanobacterial biofilm were characterized by Di Pippo et al. [21].

Since growth, as well as production of EPS by these microorganisms are significantly affected by irradiance and temperature of the culture [22–24], optimization for growth and EPS production is necessary for their mass cultivation. Temperature is one of the most important environmental parameters for growth of cyanobacteria [25]. Also, the influence of irradiance or a combination of both factors significantly regulates growth of these organisms [26,27]. A study on *Phormidium* sp. [28], showed optimum growth at a higher temperature (35°C) and a lower irradiance (26 W m⁻²), and also at a low temperature (30°C) and high irradiance (37 W m⁻²). However, a combination of high temperature and high irradiance proved inhibitory for its growth [28]. In general, cyanobacterial growth and EPS production vary with changes in temperature and light intensity, and this was reported to be species specific [29,30]. Thus, to assess the optimum temperature and irradiance requirements for growth and EPS production in cyanobacteria, experiments in crossed gradients of temperatures and irradiance are recommended. They can reveal differences in the requirements for growth and EPS production, as was demonstrated in the green alga *Dictyosphaerium chlorelloides* [31].

Two cyanobacterial species, *Scytonema tolypothrichoides* strain VB61278 and *Tolypothrix bouteillei* strain VB61268, isolated from soil crusts in India were found to produce a

PRACTICAL APPLICATION

Two cyanobacterial species, *Scytonema tolypothrichoides* and *Tolypothrix bouteillei*, isolated from soil crusts of Santiniketan, India, produce extracellular polysaccharides (EPS) in significant amounts. The EPS bind soil particles and prevent soil erosion, as well as contribute to the growth of other microorganisms and also to nutrient mobilization leading to increased soil fertility. Their application in the field should improve soil properties and increase productivity. Optimum and limiting conditions should therefore be determined for inoculum preparation and timing of its use in the field. Since EPS may also have biotechnological applications, for example, in the food or pharmaceutical industries, cultivation protocols based on growth temperature could be developed for local industry.

distinct mucilaginous sheath layer. In this study, we evaluated the effects of various levels of irradiance and temperature in crossed gradients in an experimental design created to determine the optimum temperature and irradiance conditions for maximum growth and EPS production in both cyanobacterial species.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Scytonema tolypothrichoides and *Tolypothrix bouteillei* were isolated from soil crusts of Santiniketan, Eastern India (23°40'53'' N, 87°40'22'' E) and identified according to Komárek [32]. The area falls within the tropical zone and the temperature of the upper soil surface can exceed 45°C in summer (April–June). The cyanobacteria species remain dormant in the soil throughout the year and appear upon onset of the monsoon season (July–August). Stock cultures were grown in an axenic state at 22°C and 11 W m⁻² (Osram DULUX L 55W/12-950, Germany).

2.2 | Crossed gradient experimental setup

A unit for crossed gradients of temperature and irradiance comprising 20 glass petri dishes of 9 cm diameter was used for cultivation (LABIO Co. Ltd., Prague, Czech Republic, see detailed description in Kvíderová and Lukavský [33], Supporting Information 1). A thick aluminum plate (60 × 80 × 4 cm) was cooled on one long side and heated on the opposite side. A stable temperature was controlled by pulsing energy into the heater, resulting in a precise, stable, and linear

FIGURE 1 Changes in dry weight (A, B) and EPS content (C, D) of *S. tolyporthrichoides* (A, C) and *T. bouteillei* (B, D) in crossed gradients of temperature and irradiance based on mean ($n = 5$ in each group) dry weights for each combination of these experimental variables. The data were smoothed using the distance-weighted least squares method

TABLE 1 Matrix of combinations of mean temperatures and mean irradiances used during the experiment

$I_{\max}$	$I4: 18.9 \text{ W m}^{-2}$	$T5 \times I4$	$T4 \times I4$	$T3 \times I4$	$T2 \times I4$	$T1 \times I4$
	$I3: 8.4 \text{ W m}^{-2}$	$T5 \times I3$	$T4 \times I3$	$T3 \times I3$	$T2 \times I3$	$T1 \times I3$
	$I2: 4.8 \text{ W m}^{-2}$	$T5 \times I2$	$T4 \times I2$	$T3 \times I2$	$T2 \times I2$	$T1 \times I2$
$I_{\min}$	$I1: 3.7 \text{ W m}^{-2}$	$T5 \times I1$	$T4 \times I1$	$T3 \times I1$	$T2 \times I1$	$T1 \times I1$
		$T5: 36.3^{\circ}\text{C}$	$T4: 30.3^{\circ}\text{C}$	$T3: 24.7^{\circ}\text{C}$	$T2: 17.6^{\circ}\text{C}$	$T1: 11.8^{\circ}\text{C}$
		$T_{\max}$				$T_{\min}$

$I_{\max}$, maximum irradiance; $I_{\min}$, minimum irradiance; $T_{\max}$, maximum temperature; $T_{\min}$, minimum temperature.

temperature gradient. The temperature was measured using a resistance thermometer equipped with a bead thermistor sensor (Gryf 310, Czech Republic). Water was placed between the plate surface and the bead to improve contact. Also, a wet gauze was spread under dishes over the entire plate area to improve heat transfer between the dishes and plate. A polyethylene sheet, with openings, was placed over the dishes and wet gauze to reduce evaporation. The gradient of light was perpendicular to the temperature gradient using photosynthetic active radiation through a white LED panel

(Figure 1). The intensity of irradiance was measured by a PU550 light meter equipped with a custom-made photosynthetic active radiation light sensor (Metra Blansko, Czech Republic) and the generated heat was removed by a fan and by an Infra red (IR) filter in cuvettes filled with 2 cm of water. Decreased irradiance at the sides was partly compensated by using reflective aluminum foil on both sides. The range of temperature and irradiance in the setup was 8–40°C and 3–21 Wm⁻², respectively. The final matrix of temperature × irradiance combinations is shown in Table 1. The cultivation plate

was covered by a transparent lid to reduce evaporation. In order to prevent carbon limitation, CO₂ was supplied from a CO₂ cylinder to achieve a final concentration of 2% v/v in the cultivation space. The petri dishes were shaken three times a day.

2.3 | Biomass estimation

Suspensions (1 mL) of homogenized cyanobacterial cells ($A_{750} = 1.0$) were inoculated into each petri dish containing 19 mL of BG-11 medium (without nitrogen), corresponding to an initial dry weight (DW) of 0.063 mg mL⁻¹ and 0.055 mg mL⁻¹ for *S. tolypothrichoides* and *T. bouteillei*, respectively. After 25 days (over 500 h) growth, cyanobacterial cultures were harvested and centrifuged. Five replicates were taken from each temperature–irradiance combination. The cell pellets were resuspended in distilled water. The cells were washed three times with distilled water, filtered on preweighed filter discs, and dried for 24 h at 110°C to reach constant weight.

2.4 | EPS estimation

The initial EPS content was below the detection limit of the method and instrumentation used, that is, below 100 µg mL⁻¹. Three replicates were taken from each temperature–irradiance combination after 25 days of cultivation. The carbohydrate content was estimated using anthrone reagent [34], prepared in an amber bottle by dissolving 100 mg of anthrone in 100 mL of 75% v/v sulphuric acid. The mixture was kept in a water bath at 85°C to dissolve the anthrone completely. Homogeneous suspensions of cells in stationary phase (0.2 mL) were sampled and diluted five times with distilled water making a final volume of 1 mL. The diluted cell suspensions were centrifuged at 3500 rpm for 20 min. Each supernatant was placed into another test tube for estimation of extracellular carbohydrate (EPS). Anthrone reagent (4 mL) was added to each test tube. Tubes were kept in a boiling water bath for 15 min by covering the mouth of each tube with aluminum foil to avoid evaporation. The tubes were then cooled to ambient temperature. Absorbance at 620 nm (A_{620}) was read in a Systronics 105 spectrophotometer against a reference containing 1 mL of distilled water and 4 mL of anthrone reagent. Carbohydrate content was estimated using a glucose standard.

2.5 | Data normalization

For optima comparisons, DW and EPS datasets for both strains were normalized to dataset minima and maxima according to the following equation:

$$NV = \frac{AV - \min}{\max - \min},$$

where NV is the normalized value of a given data point; AV, the actual (real) value of a given data point; min, the minimum value in the dataset; and max, the maximum value in the dataset.

Datasets included 100 cases and 60 cases for the DW and EPS content, respectively, in 20 temperature × irradiance combinations (Table 1). For determination of the temperature optima, datasets consisted of 25 cases and 15 cases for DW and EPS content, respectively, at each irradiance level. A total of four irradiance levels were considered (Table 1). For determination of the irradiance optima, datasets consisted of 20 cases and 12 cases for DW and EPS contents, respectively, at each temperature level. A total of five temperature levels were considered (Table 1).

2.6 | Statistical analyses

The statistical analyses were performed using Statistica 13.0 [35] and Canoco 5.0 [36] softwares.

The null hypotheses stated there were no effects of temperature, irradiance, or their combinations on (A) growth rates of the experimental strains, (B) on EPS production of the experimental strains, and (C) there were no differences in growth and EPS production requirements.

Two-way analysis of variance (ANOVA) and one-way ANOVA + Tukey honest significant difference test (HSD) were used to evaluate the effects of temperature, irradiance, and their combination on DW and EPS content. The Redundancy Analysis (RDA) with the Monte Carlo Permutation test was used to determine the significance of effects of temperature and irradiance on growth and EPS production. Generalized Additive Models (GAMs) were used to plot differences in the effects of temperature and irradiance on growth and EPS production in experimental organisms. The differences were considered statistically significant at $p < 0.05$.

3 | RESULTS

3.1 | Optimum temperature and irradiance requirements for growth of *S. tolypothrichoides* and *T. bouteillei*

The final DW of *S. tolypothrichoides* was significantly affected by temperature ($n = 100$, $F = 2135$, $p < 0.001$), irradiance ($n = 100$, $F = 588.4$, $p < 0.001$), and their combinations ($n = 100$, $F = 25.13$, $p < 0.001$). Optimum growth was observed in the temperature range of 27 to 34°C and irradiance range of 9 to 13 W m⁻². Minimum DW was observed at lower temperatures, but *S. tolypothrichoides* was sensitive to higher irradiances in the suboptimum temperature range (12–24°C); 40°C was the upper limit for growth and the organism did not tolerate very high or low irradiances (Figure 1A).

The growth temperature optimum of ca. 30°C remained stable at different irradiances (Figure 2, Supporting Information 2), while the irradiance optimum was shifted from ca. 9 W m⁻² at temperatures between ca. 25 and 36°C to ca. 5 W m⁻² at 18°C. The decline in optimum irradiance could be due to decreased rates of photosynthetic processes at low temperatures. The decline in DW indicated that low irradiance was the limiting factor for growth at a particular temperature. In contrast, high irradiances exceeded growth saturation values and led to partial photoinhibition (Supporting Information 2 and 3).

The final DW of *T. bouteillei* in crossed gradients was significantly affected by temperature ($n = 100$, $F = 713.2$, $p < 0.001$), irradiance ($n = 100$, $F = 153.1$, $p < 0.001$), and their combinations ($n = 100$, $F = 25.13$, $p < 0.001$). Optimum growth was observed within the temperature range of 22 to 32°C and the irradiance range of 7 to 18 W m⁻². The lower temperature growth limit of 12°C was similar to *S. tolypothrichoides*, however, *T. bouteillei* was less sensitive to marginal changes in irradiances; the upper growth limit was slightly higher than in *S. tolypothrichoides* (Figure 1B).

At the lowest irradiance, the temperature optimum was shifted slightly to a higher temperature (Figure 3, Supporting Information 2). An irradiance optimum was not identified at 12, 18, and 36°C and there was no difference in irradiance optima at 25 and 30°C (Supporting Information 2 and 4). The optimum growth temperature of *S. tolypothrichoides* (27–34°C) was higher than in *T. bouteillei* (22–32°C). The optimum growth irradiance of *S. tolypothrichoides* (9–13 W m⁻²) was slightly lower than in *T. bouteillei* (7–18 W m⁻²) (Figures 1–3, Supporting Information 2–4).

3.2 | Optimum temperature and irradiance requirements for polysaccharide production by *S. tolypothrichoides* and *T. bouteillei*

The EPS content of *S. tolypothrichoides* was affected significantly by temperature ($n = 60$, $F = 1019$, $p < 0.001$), irradiance ($n = 60$, $F = 314.1$, $p < 0.001$), and their combinations ($n = 60$, $F = 168.8$, $p < 0.001$). The optimum temperature for EPS production ranged from 28 to 34°C and the optimum irradiance ranged from 8 to 13 W m⁻². These values corresponded well with the growth optimum. The EPS content increased at suboptimal temperatures, however, at supraoptimal temperatures, it declined compared to growth. In suboptimal irradiances, the increase in EPS content with increasing irradiance was higher. A very low EPS content was detected under marginal conditions (Figure 1C). No shift in the irradiance optimum for EPS production was observed in the temperature gradient (Figure 4, Supporting Information 2). At optimal and supraoptimal irradiances, the EPS content

remained comparable to the optimum value at suboptimal growth temperatures (Supporting Information 2 and 5), while it was slightly decreased in optimal and supraoptimal ones.

EPS production in *T. bouteillei* was also affected by temperature ($n = 60$, $F = 1049$, $p < 0.001$), irradiance ($n = 60$, $F = 46.86$, $p < 0.001$), and their combinations ($n = 60$, $F = 57.16$, $p < 0.001$). The temperature optimum for EPS production in this organism ranged from 27 to 34°C, which was slightly higher than the optimum for DW, while the irradiance optimum for EPS was 7 to 17 W m⁻².

The increased production of EPS at higher temperatures might be part of an adaptation/acclimatization mechanism to heat/desiccation stress encountered in its normal niche. In *S. tolypothrichoides*, an increase in EPS content was gradual at suboptimal temperatures but under supraoptimal conditions its decrease was sudden (Figure 1D). No shift in the temperature optimum was observed in the irradiance gradient and there was no change in EPS content at their radiance optimum (Figure 5, Supporting Information 2 and 6). The maximum EPS content was detected over similar temperature ranges (27/28–34°C) in both strains. The optimum irradiance was slightly lower (considering the mean) and the range narrower in *S. tolypothrichoides* (8–13 W m⁻²) than in *T. bouteillei* (7–17 W m⁻²) (Figures 1–5, Supporting Information 3–6).

3.3 | Redundancy analysis and generalized additive models

Although the RDA analysis did not find any effects of temperature and irradiance on DW and EPS content in either strain of cyanobacteria (total variation = 5.22, explained variation = 19.7%; Monte Carlo permutation test on the first ordination axis pseudo- $F = 4.0$, $p = 0.108$; Monte Carlo permutation test on all ordination axes pseudo- $F = 2.1$, $p = 0.112$), temperature was found to be the main driving factor (total variation = 5.22, explained variation = 18.8%; Monte Carlo permutation test on all ordination axes pseudo- $F = 4.2$, $p = 0.038$). The effect of irradiance itself was found to be negligible and nonsignificant (total variation = 5.22, explained variation = 1.4%; Monte Carlo permutation test on all ordination axes pseudo- $F = 0.3$, $p = 0.786$).

The GAM confirmed differences in requirements for growth and EPS production in the two experimental cyanobacterial strains. *Scytonema tolypothrichoides* preferred a higher temperature and a lower irradiance than *T. bouteillei*. The quantity of EPS was higher at slightly increased temperatures compared to the growth optimum, reflecting its possible role in adaptation/acclimatization to heat/desiccation stress (Figure 6). The GAM confirmed a nonsignificant effect of irradiance (Supporting Information 7).

FIGURE 2 The shift in dry weight (A) and normalized dry weight (B) temperature optima at individual irradiance levels, indicated by the value in the chart for *S. tolypothrichoides* (mean $\pm$ SD, $n = 5$ in each group). The data were normalized to minimum and maximum values at a given temperature or irradiance. The letters indicate the homologous groups recognized by the Tukey HSD test at $p = 0.05$

4 | DISCUSSION

Soil cyanobacteria play an important role in soil stabilization and fertilization in the arid areas of tropical and subtropical regions. Novel methods based on utilization of local cyanobacterial strains for maintenance of soil stability and soil nutrient content are crucial for the development of sustainable agriculture in these regions, and for reducing the effects of ongoing global climate change. For the first time, growth and EPS production for two local cyanobacterial strains were evaluated.

4.1 | Growth requirements

The temperature requirements of *S. tolypothrichoides* VB61278 and *T. bouteillei* VB61268 indicated that both were

mesophilic [37]. Although these cyanobacteria were capable of surviving short-term exposure to 45°C, the optimum temperature for long-term growth did not exceed 35°C. The temperature optima for both species reflects an adaptation to temperature in their natural habitats, where short-term exposure to high temperatures of 35°C and occasionally even higher occurs in a tropical country like India. Their acclimation to culture conditions, that is, 22°C [38] cannot be excluded completely. However, the temperature optima obtained in this study corresponded to common optima of tropical soil cyanobacteria [39]. Higher growth at elevated temperatures supports the hypothesis that the experimental species maintained their temperature requirements, which are acquired at the original locality.

The irradiance requirements indicate an adaptation to relatively low irradiances, which is typical for shaded localities

FIGURE 3 The shift in dry weight (A) and normalized dry weight (B) temperature optima at individual irradiance levels, indicated by the value in the chart for *T. bouteillei* (mean $\pm$ SD, $n = 5$ in each group). The data were normalized to minimum and maximum values at a given temperature or irradiance. The letter indicates the homologous groups recognized by the Tukey HSD test at $p = 0.05$

like soil [40] or lower layers of soil crust [26,41]. Since an irradiance of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ ($\sim 10.9 \text{ W m}^{-2}$, recalculated according to Ref. 42 in culture) is similar to expected irradiance encountered in situ, the selection pressure was not strong, and the species maintained their optimum irradiance requirements.

Despite their origin from the same microhabitat, the temperature and irradiance requirements of the species differed. *Scytonema tolypothrichoides* preferred higher temperatures and lower irradiances than *T. bouteillei*, suggesting that each species inhabits slightly different niches in the natural biocrust. Slight differences in growth optima of cyanobacterial species originating from the same locality were reported from Tunis and Slovakia [43]. The occurrence of this phenomenon across different climatic zones indicates

that the slight difference in growth optima of cyanobacteria and algae from the same site may be common in nature [34]. In environments with low temperature variation, the species-specific temperature optima might be similar, even the same, as in an environment with high temperature variability.

The data presented here indicate that for preparation of an inoculum for field application, slightly different temperature and irradiance conditions should be applied for each strain to achieve maximum DW production. As both experimental strains seem to be mesophilic and low-irradiance adapted, these requirements could be achieved easily in mass cultivation for inoculum production under local conditions. With respect to the local climate, active cooling of the cultivation might be required and the mass cultivation units should be screened from full sunlight. Active monitoring of the

FIGURE 4 The shift in EPS content (A) and normalized EPS content (B) temperature optima at individual irradiance levels, indicated by the value in the chart for *S. tolypothrichoides* (mean $\pm$ SD, $n = 3$ in each group). The data were normalized to minimum and maximum values at a given temperature or irradiance. The letter indicates the homologous groups recognized by the Tukey HSD test at $p = 0.05$

physiological status of these cultures using the variable chlorophyll fluorescence approach should be beneficial for controlling growth conditions [44,45].

For maximum EPS production in the field, the conditions described above should be used to achieve a dense culture in stationary phase prior to application of the inoculum. Our data could help in the development of a reliable protocol for commercial applications, either for improving soil properties or as the first stage in the production of biotechnologically valuable compounds.

4.2 | EPS production

Synthesis and accumulation of EPS play an important role in stress responses to desiccation [2,3,46,47], freezing [47,48], and water stress [49,50]. Such stress tolerance mechanisms

seem to be evolutionally old, since it has been observed in various organisms with different degrees of complexity, for instance, bacteria [2,51,52] and higher plants [53,54]. The production of various types of extracellular matrix (mucilaginous envelopes, capsules or sheaths) has also been reported to be the mechanisms of desiccation tolerance, together with synthesis of osmotically active compounds such as sugars in other cyanobacterial species [2,3,55].

Since desiccation is usually connected with elevated temperatures and/or increased irradiance in nature, higher amounts of EPS are synthesized and accumulated at higher temperatures as an adaptation to expected desiccation. Indeed, increased contents of EPS in *S. tolypothrichoides* and *T. bouteillei* were observed in this study at higher temperatures. EPS production was also increased at higher temperatures in *Anabaena* sp. [22]. However, the effect of temperature

FIGURE 5 The shift in EPS content (A) and normalized EPS content (B) temperature optima at individual irradiance levels, indicated by the value in the chart for *T. bouteillei* (mean $\pm$ SD, $n = 3$ in each group). The data were normalized to minimum and maximum values at a given temperature or irradiance. The letter indicates the homologous groups recognized by the Tukey HSD test at $p = 0.05$

FIGURE 6 GAM charts indicating the effect of temperature (A) and irradiance (B) on dry weight and EPS content

is species-specific, since higher temperatures decreased EPS production in *Spirulina* sp. [56] but temperature did not affect EPS synthesis in *Nostoc* sp. [6,57]. The optimum temperature for EPS production in *S. tolypothrichoides* and *T. bouteillei* was approximately 30 to 35°C, comparable to *Arthrospira platensis* [24]. CCA analysis showed that in both experimental organisms, maximum EPS production occurred at slightly higher temperatures than optimum growth. Such a difference indicates that other abiotic and biotic factors may also influence total EPS production [8,11,58].

High and continuous irradiance increased significantly EPS production in cyanobacteria [58]. The preference for higher irradiance for EPS production than for growth was found in *S. tolypothrichoides*. In contrast, in *T. bouteillei*, maximum EPS content was achieved at lower irradiances than maximum DW. As in the case of temperature effects, the irradiance optimum for EPS production may differ from the growth optimum, reflecting the influence of other experimental factors on EPS production [8,11,58].

As the required EPS production should occur in the field, long-term weather statistics and actual weather forecasts for at least 1 week must be considered before application of an inoculum. For field application, the inoculum will be prepared in liquid medium in large-scale cultivation units (e.g. ponds) and applied by spraying, either from the ground (water carts, irrigation system) or the air (powdering aircrafts). Therefore, the conditions in the mass cultivation should be focused on maximum production of biomass, and the application in the field should occur at conditions either supporting further growth followed by those for maximum EPS production or inducing the production of the EPS almost immediately. The inoculum should be applied on bare soil in a time period between the harvest and sowing. Our data will help to upscale the mass cultivation, to setup the transport conditions to the field and inoculum storage before application, and to determine the timing of field applications with respect of actual weather forecast. For maximum EPS content as the final product, knowledge of the conditions for maximum EPS production is necessary, and our data could help in the development of a protocol for industrial applications.

5 | CONCLUSIONS

The results of our study indicate that optimum conditions for growth and polysaccharide production are species specific and may differ in response to local environmental conditions. Since previous studies were usually performed under optimum or near-optimum growth conditions, the real optimum for EPS production was not clear [58]. Cultivation in crossed gradients of temperature and irradiance represents a precise approach to resolve this problem.

Therefore, we propose a two-step strategy for growing biomass for field applications: (1) grow the biomass for soil inoculation under controlled conditions in mass cultures, and (2) apply a dense inoculum in the field, taking account of expected weather conditions to achieve maximum EPS production in situ. When considering the EPS as biotechnologically valuable compounds, our data could also be beneficial in developing a two-stage production protocol.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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