



Supplementation of *Aspergillus glaucus* with *gfdB* gene encoding a glycerol 3-phosphate dehydrogenase in *Aspergillus nidulans*

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Abstract

In *Aspergillus nidulans*, there are two putative glycerol 3-phosphate dehydrogenases encoded by the genes *gfdA* and *gfdB*, while the genome of the osmophilic *Aspergillus glaucus* harbors only the ortholog of the *A. nidulans* *gfdA* gene. Our aim was to insert the *gfdB* gene into the genome of *A. glaucus*, and we reached this goal with the adaptation of the *Agrobacterium tumefaciens*-mediated transformation method. We tested the growth of the *gfdB*-complemented *A. glaucus* strains on a medium containing 2 mol l⁻¹ sorbitol in the presence of oxidative stress generating agents such as *tert*-butyl hydroperoxide, H₂O₂, menadione sodium bisulfite, as well as the cell wall integrity stress-inducing agent Congo Red and the heavy metal stress eliciting CdCl₂. The growth of the complemented strains was significantly higher than that of the wild-type strain on media supplemented with these stress generating agents. The *A. nidulans* Δ *gfdB* mutant was also examined under the same conditions and resulted in a considerably lower growth than that of the control strain in all stress exposure experiments. Our results shed light on the fact that the *gfdB* gene from *A. nidulans* was also involved in the stress responses of the complemented *A. glaucus* strains supporting our hypothesis on the antioxidant function of GfdB in the Aspergilli. Nevertheless, the osmotolerant nature of *A. glaucus* could not be explained by the lack of the *gfdB* gene in *A. glaucus*, as we hypothesized earlier.

KEYWORDS

Aspergillus glaucus, *Aspergillus nidulans*, *Agrobacterium*-mediated transformation, glycerol 3-phosphate dehydrogenase, oxidative stress

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1 | INTRODUCTION

Gene duplications are of outstanding importance in the evolution of fungi [1–5]. Nevertheless, increased gene quantities may be disadvantageous for the organism because surplus genes may interfere with normal cell physiological functions [4,6]. However, subsequent neofunctionalisation and subfunctionalization mechanisms may stabilize both copies of the duplicated genes, and hence, may also protect the fungus from the negative consequences of imbalanced gene expressions [5,7]. For instance, in *Aspergillus nidulans* two genes, *gfdA* and *gfdB*, putatively encode glycerol 3-phosphate dehydrogenases with some shared but also with some quite different physiological functions [8,9]. Not surprisingly, these gene functions seem to be largely complementary to each other [9]. Based on stress tolerance assays, the gene product GfdA is mainly involved in the osmotic stress tolerance of *A. nidulans*, meanwhile, the GfdB isozyme is an important element of oxidative stress defense system of this filamentous fungus [8,9]. Importantly, in the genomes of *Aspergillus glaucus* and *Aspergillus wentii*, *gfdA* is present exclusively and *gfdB* is absent, which might be related to the observed osmophilicity of these Aspergilli [10]. To unfold the role of GfdB, for example, in the stress tolerance of the Aspergilli, we introduced the *gfdB* gene into the genome of *A. glaucus* to compensate for the lack of any *gfdB* ortholog. In these genetic complementation experiments, we adapted the *Agrobacterium*-mediated transformation method described previously for the industrial fungus *Claviceps paspali* by Kozák et al. [11]. With the *A. nidulans gfdB*-complemented *A. glaucus* strains at our disposal, we screened and compared the stress tolerances of wild-type control *A. nidulans* and *A. glaucus* strains and also those of the $\Delta gfdB$ *A. nidulans* gene deletion and the 'c *gfdB* *A. glaucus* gene complemented strains [9,12].

2 | MATERIALS AND METHODS

2.1 | Strains, culture media, and production of conidia

The following strains were used in our study: *A. nidulans* THS30.3 (*pyrG89*, *AfupyrG*⁺; *pyroA*⁺; *veA*⁺; prototrophic control) and $\Delta gfdB$ (*pyrG89*; $\Delta gfdB::AfupyrG$ ⁺; *pyroA*⁺; *veA*⁺) [9] strains, and *A. glaucus* CBS516.65 (kindly provided by Prof. Dr. Ronald de Vries, Westerdijk Fungal Diversity Institute, Utrecht, The Netherlands) and 'c *gfdB1* and 'c *gfdB2 gfdB*-complemented strains.

The *A. nidulans* strains were maintained on Malt Extract Agar (MEA), and MEA agar plates were

incubated at 25°C for 6 days [13]. The *A. glaucus* strains were cultured on MEA also supplemented with 0.5 mol l⁻¹ NaCl, and the plates were incubated at 25°C for 6 days [10,12]. Conidia harvested from these 6-day-old plates were used in all stress tolerance screening experiments.

2.2 | Construction of the *A. nidulans gfdB*-complemented strains of *A. glaucus*

The plasmid pAg-H3 (Figure S1) was used to transform *A. glaucus* by *Agrobacterium tumefaciens*-mediated transformation. The pAg-H3 plasmid and the *A. tumefaciens* LBA4404 strain were kindly provided by Prof. Dr. István Molnár, Southwest Center for Natural Products Research, the University of Arizona, Tucson, AZ. The *gfdB* gene encoding a putative glycerol 3-phosphate dehydrogenase (locus ID: AN6792; for more information visit the AspGD database [http://www.aspergillusgenome.org/cgi-bin/locus.pl?locus=GfdB&organism=A_nidulans_FGSC_A4]; [10]) with its native promoter and terminator sequences (for primer pair see Table S1) was cloned to the *Sma*I–*Aw*rII site of the pAg-H3 plasmid and, following that, the plasmid was transformed into *A. tumefaciens* cells by electroporation.

The *A. tumefaciens*-mediated transformation of *A. glaucus* was carried out following the protocol of Kozák et al. [11] with some modifications. *A. glaucus* mycelia were collected from the surface of MEA supplemented with 0.5 mol l⁻¹ NaCl and were suspended in 5 ml distilled water, then inoculated into 50 ml artificial seawater [14] in a 500 ml Erlenmeyer flask and were cultivated for 48 h at 28°C with shaking at 3 Hz frequency. A 5 ml aliquot of this preculture was inoculated into 50 ml fresh artificial seawater in a 500-ml Erlenmeyer flask and was cultured for an additional 24 h at 28°C at 3 Hz shaking frequency. Mycelia were collected by centrifugation for 5 min at 2,000 g, washed in 40 ml of distilled water, then suspended at 100 mg ml⁻¹ in wet cellular biomass concentration in induction medium (IM) broth [15] and also supplemented with 200 µmol l⁻¹ acetosyringone to induce *A. tumefaciens* infection, and were incubated for another 8 h at 28°C with shaking (3 Hz shaking frequency).

The cultivation of *A. tumefaciens* cells was carried according to Kozák et al. [11]; 100 µl aliquots of the *A. glaucus* mycelia and 75 µl aliquots of the *A. tumefaciens* cells were mixed, spread onto IM agar plates containing 200 µmol l⁻¹ acetosyringone [15], and the coculture was incubated for 6 days at 28°C. To select transformants and inhibit the further growth of the *A. tumefaciens* cells, the IM agar plates were overlaid with 10 ml of top agar (IM containing 0.5 mol l⁻¹ NaCl and also supplemented with 150 µg ml⁻¹ hygromycin and 600 µg ml⁻¹ cefotaxime), and the plates were kept at 28°C for 10 days. The hygromycin-resistant colonies were

inoculated onto MEA plates containing 200 $\mu\text{g ml}^{-1}$ hygromycin and 600 $\mu\text{g ml}^{-1}$ cefotaxime. The outgrowing colonies were incubated at 28°C for 7 days, and reisolation was repeated at least four times.

2.3 | Copy number analysis of the *gfdB* gene using the quantitative polymerase chain reaction method

Six serial 1:2 dilutions (320, 160, 80, 40, 20, and 10 ng, respectively, in 7 μl nuclease-free water) of genomic DNA isolated from the *gfdB*-complemented strains of *A. glaucus* were used to generate standard curves of the C_T (threshold cycle) value for the *gfdB* gene against the log DNA concentration on each polymerase chain reaction (PCR) plate. *Aspg1_0039306* (encoding the *A. nidulans* γ -glutamylcysteine synthetase ortholog in *A. glaucus*) was used as a single-copy reference gene. Each experiment was repeated three times [16].

Quantitative PCR was performed in a total volume of 20 μl , which was composed of 10 μl Fast SYBR® Green master mix (Applied Biosystems by Life Technologies), 0.4 μl reverse primer, 0.4 μl forward primer (the primers are listed in Table S1), 7 μl diluted genomic DNA, and 2.2 μl nuclease-free water. PCR cycles were performed according to the following protocol: 95°C 2 min; 40 cycles: 95°C 5 s, 51°C 10 s, 65°C 20 s; 95°C 15 s, 51°C 15 s, 95°C continuous, 37°C 1 s.

Equation (1) from the Equation (2) for a line was constructed by plotting the standard curve of the log quantity versus its corresponding C_T value

$$C_T = m(\log \text{ quantity}) + b, \quad (1)$$

$$y = mx + b. \quad (2)$$

If the curve demonstrated an $r^2 > .980$, the standard curve was then used to determine the sensitivity, primer efficiencies, and the dynamic range as well as the specificity and reproducibility of each assay. The copy numbers of the *gfdB* gene were determined by the absolute quantitation method, by which total copies were first calculated using the following equation:

$$gfdB \text{ copies} = 10^{([C_T] - b/m)}. \quad (3)$$

The number of *gfdB* copies per genome was then determined by the following equation [16]:

$$gfdB \text{ copies per genome} = (\text{total copies of } gfdB) / (\text{total copies of } Aspg1_0039306). \quad (4)$$

2.4 | Determination of the *gfdB* gene expression by real-time reverse-transcription PCR assay in *A. glaucus* 'c *gfdB*-complemented strains

For RNA isolation, mycelia from surface cultures (untreated as well as *tBOOH* treated) were collected after 4 days and stored at -80°C . Total RNA was extracted from freeze-dried mycelial mats using TRIzol reagent [17]. Real-time polymerase chain reactions with QuantiTect™ SYBR® Green rRT-PCR Kit (Qiagen, Germany) were carried out according to the manufacturer's recommendations with 500 ng of total RNA per reaction in 40 cycles. The steps for the real-time reverse-transcription (rRT)-PCR reaction were as follows: (a) reverse-transcription: 50°C, 30 min; (b) PCR initial activation step: 95°C, 15 min; (c) DNA denaturation: 94°C, 15 s; (d) annealing: T_m : 5–8°C, 30 s; (d) extension: 72°C, 30 s and 40 cycles. In each RNA sample, *Aspg1_0030273* [*tif1* (*Afu3g08160*) *A. fumigatus* ortholog] transcripts were also quantified as reference gene transcripts (Table S1) [18]. Relative transcript levels were calculated by the “delta method” where $\Delta C_T = C_{T \text{ reference gene}} - C_{T \text{ gfdB}}$ and C_T stands for the rRT-PCR cycle numbers corresponding to the crossing points. For statistical analysis, the mean \pm standard deviation (*SD*) values were calculated from three independent experiments [19,20]. Relative transcript levels were examined using the following reference genes as well: *Aspg1_0036969* (*A. fumigatus* *fsk1* ortholog), *Aspg1_0140429* (*A. fumigatus* *tef1* ortholog), *Aspg1_0027831* (*A. nidulans* *actA* ortholog), *Aspg1_0051175* (*A. nidulans* AN6700 ortholog) with similar results.

2.5 | Stress tolerance studies

To study and compare the stress sensitivities of the *A. nidulans* control and $\Delta gfdB$ gene deletion strains as well as those of the *A. glaucus* control and the 'c *gfdB1* and 'c *gfdB2* complemented strains, the stress agar plate assays of Balázs et al. [13] were adapted. The following stress generating agents were tested on Barratt's nitrate minimal medium (NMM) [21]: oxidative stress: 9 mmol l^{-1} hydrogen peroxide, 1.5 mmol l^{-1} diamide, 0.4 (*A. glaucus*) and 0.8 (*A. nidulans*) mmol l^{-1} *tert*-butyl hydroperoxide, 0.096 (*A. glaucus*) and 0.19 (*A. nidulans*) mmol l^{-1} menadione sodium bisulphite (MSB); hyperosmotic stress: 2 mol l^{-1} sorbitol, 1 mol l^{-1} NaCl; cell wall integrity stress: 54 $\mu\text{mol l}^{-1}$ Congo Red; heavy metal stress: 0.1 (*A. glaucus*) and 0.3 (*A. nidulans*) mmol l^{-1} cadmium chloride. In the case of *A. glaucus*, these stress agents were tested on minimal media supplemented with 2 mol l^{-1} sorbitol or

1 mol l⁻¹ NaCl, respectively. Stress agar plates were point-inoculated with 5 µl aliquots of freshly made conidia suspensions (2 × 10⁷ conidia ml⁻¹) and were incubated at 25°C for 5 and 10 days [10,12,13]. Diameters of the colonies were measured and used for the characterization of the stress tolerances of the strains. In the stress tolerance studies with the *A. nidulans* Δ *gfdB* strain, the isogenic prototrophized THS30.3 strain was used as control.

2.6 | Statistical analysis of experimental data

All experiments were performed in three independent sets, and mean ± SD values were calculated and are presented. Statistical significances were calculated using Student's *t*-test, and *p* < .05 were considered as statistically significant and shown in the following form: **p* < .05, ***p* < .01, and ****p* < .001 or #*p* < .05, ##*p* < .01, and ###*p* < .001.

3 | RESULTS

To gain more information on the physiological functions of *gfdB* in the Aspergilli in general, we constructed *A. nidulans* *gfdB*-complemented *A. glaucus* strains ('*c gfdB1*' and '*c gfdB2*'). For the genetic modification of *A. glaucus*, we adapted the *Agrobacterium*-mediated transformation method using the pAg-H3 plasmid containing the *gfdB* gene with its native promoter and terminator sequences (Figure S1) [11] to introduce the *gfdB* gene into the *A. glaucus* genome.

Before the planned extensive stress tolerance assays, we demonstrated using quantitative PCR protocols that both *gfdB*-complemented *A. glaucus* strains—'*c gfdB1*' and '*c gfdB2*'—harbored a single copy of the *A. nidulans gfdB* gene (Table 1) [16] and we also confirmed the *gfdB* gene expression in both *A. glaucus* complemented strains using rRT-PCR, although it was not induced by the oxidative stress initiating agent *t*BOOH (0.4 mmol l⁻¹) on

stress agar plates also supplemented by 2.0 mol l⁻¹ sorbitol (Figure S2).

For phenotypic characterization, we tested the stress tolerances of the wild-type and *gfdB*-complemented *A. glaucus* strains as well as the *A. nidulans* Δ *gfdB* mutant and control strains in the presence of oxidative, cell wall integrity as well as heavy metal stress generating agents (Figures 1 and 2 and Table S2) [9,10,12,13]. In *A. glaucus*, the insertion of *gfdB* into its genome brought about significantly higher stress tolerance of the fungus when treated with the oxidative stress eliciting agents *t*BOOH, MSB and H₂O₂, with the cell wall integrity stress generating Congo Red as well as the heavy metal stress-inducing CdCl₂. These phenotypes were observed at 25°C and 10 days incubation time when 2 mol l⁻¹ sorbitol was used as an osmolyte (Figures 1 and S3–S5 and Table S2). The most interesting phenotype was observed in the presence of 0.4 mmol l⁻¹ *t*BOOH, where *gfdB* fully restored the growth of *A. glaucus*, and hence, it was comparable with the growth of the wild-type strain without any *t*BOOH treatment (Figure 1 and Table S2).

Vice versa, in *A. nidulans* the inactivation of *gfdB* decreased the stress tolerance of the fungus treated with the same stress generating agents, namely *t*BOOH, MSB, H₂O₂, Congo Red as well as CdCl₂ under the same cultivation conditions (25°C, 10 days, without any sorbitol supplementation; Figures 2, S6, and S7 and Table S2).

When *A. glaucus* strains were incubated only for 5 days at 25°C, larger colony diameters were only observed in the presence of Congo Red and MSB, and only in the case of the '*c gfdB2*' strain (Figures S8–S11).

When the same set of experiments was repeated by replacing 2 mol l⁻¹ sorbitol with 1 mol l⁻¹ NaCl as an osmotic stabilizer no differences were found in stress tolerances of the wild-type and the *gfdB*-complemented *A. glaucus* strains (Figures S12–S15 and Table S2).

According to the results of the stress sensitivity assays, insertion of the *gfdB* gene did not affect the osmophilic phenotype of *A. glaucus* in the presence of either 2 mol l⁻¹ sorbitol or 1 mol l⁻¹ NaCl since the *gfdB*-complemented *A. glaucus* strains showed similar growth to the wild-type strain in the presence of either 2 mol l⁻¹

Strains	<i>gfdB</i>	<i>r</i> ²	<i>AspgI1_0039306</i>	<i>r</i> ²	Copy number
' <i>c gfdB1</i> '	$y = -6.76x + 39.54$	1.00	$y = -6.01x + 36.99$.99	1.01 ± 0.14
' <i>c gfdB2</i> '	$y = -5.65x + 34.56$.98	$y = -5.15x + 31.61$.99	0.97 ± 0.16

Note: The *A. glaucus gfdB* copy numbers were determined by the absolute quantitation method. The number of AN6792 (*gfdB*) per genome was determined by the following equation: AN6792 (*gfdB*) per genome = (total copies of AN6792 (*gfdB*))/(total copies of *AspgI1_0039306*). The equation $C_T = m(\log \text{quantity}) + b$ from the equation for a line ($y = mx + b$) was constructed by plotting the standard curve of log quantity versus its corresponding C_T value, where $y = C_T$ value, $m = \text{slope}$, $x = \log(\text{quantity})$, $b = \text{intercept}$.

TABLE 1 Copy number determination of the complemented *Aspergillus glaucus* strain '*c gfdB1*' and '*c gfdB2*' using *AspgI1_0039306* (*Aspergillus nidulans* γ -glutamylcysteine synthetase ortholog) gene as a single copy reference gene

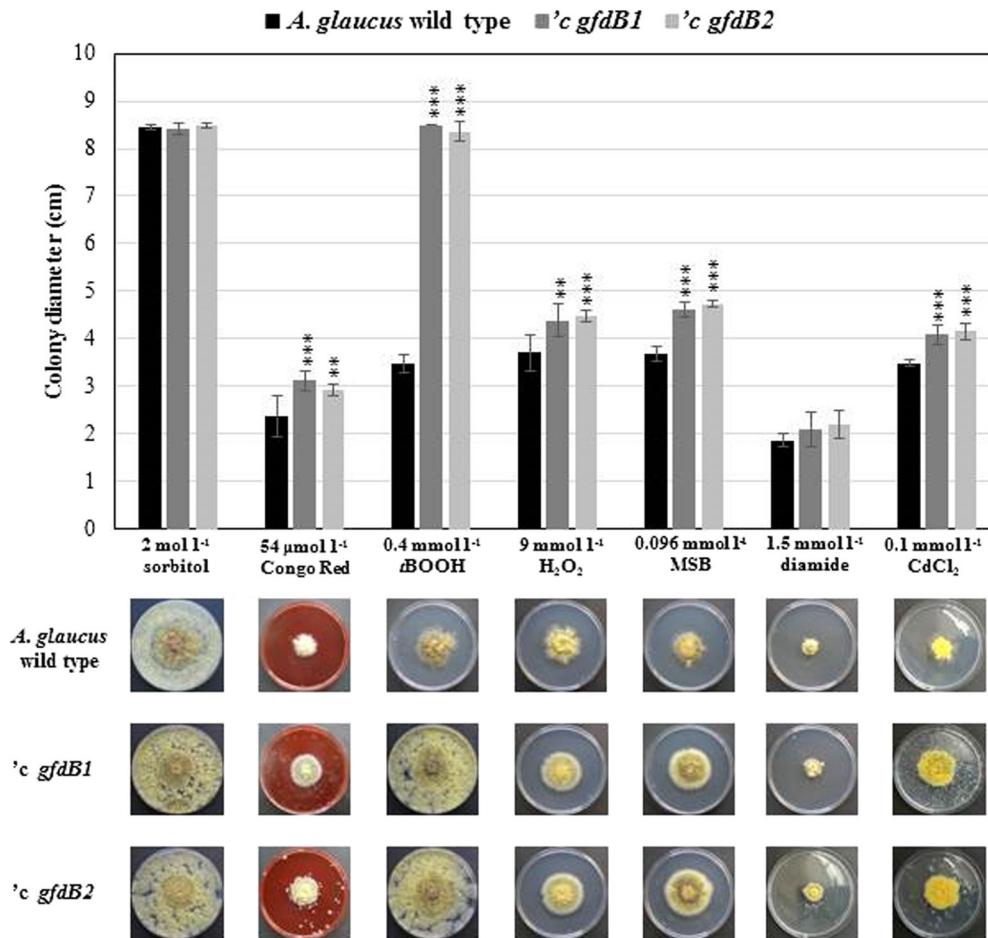


FIGURE 1 Stress tolerances of the wild-type as well as the *gfdB*-complemented '*c gfdB1* and the '*c gfdB2* *Aspergillus glaucus* strains exposed to various types of stress. Stress sensitivities observed in surface cultures on nitrate minimal medium agar plates supplemented with 2 mol l⁻¹ sorbitol are shown. Data are presented as mean ± standard deviation values calculated from three independent experiments. Plates were incubated at 25°C for 10 days. Significant differences between control and mutant cultures (**p* < .05, ***p* < .01, and ****p* < .001) are shown

sorbitol or 1 mol l⁻¹ NaCl (Figures 1, S3–S5, and S8–S15 and Table S2).

4 | DISCUSSION

Filamentous fungi can adapt adequately to a wide spectrum of abiotic and biotic stresses in their environment. For example, among Aspergilli most of the species can occupy a wide range of habitats owing to their highly efficient stress sensing, their complex and sophisticated stress signal transduction networks as well as their elaborated and robust stress defense systems [10,22]. For instance, remarkably high osmotic stress tolerance or even osmophilicity are a quite common phenomena observable in the *Aspergillus* genus, including, for example, the species *A. glaucus*, *A. wentii*, *A. versicolor*, and *A. sydowii* [10,12]. In *A. glaucus* and *A. wentii*, the

well-described osmophilic phenotypes were associated with the lack of *gfdB* (encoding a putative glycerol 3-phosphate dehydrogenase, e.g., in *A. nidulans*) and harboring merely the ortholog of the *gfdA* gene in their genomes [10].

We managed to insert the *gfdB* gene with its native promoter and terminator sequence into the *A. glaucus* genome by the adaptation of the *A. tumefaciens* transformation method and the expression of the *gfdB* gene was also proved by RT-PCR. The successful complementation of the lack of any *gfdB* ortholog in the *A. glaucus* genome increased the oxidative, cell wall integrity, and heavy metal stress tolerances of the fungus. It is also noteworthy that longer incubation times were needed at lower than 25°C incubation temperature to see the phenotypic consequences of *gfdB* insertion (*A. glaucus* does not grow at 37°C [10,12]). Considering the physiological roles attributed to the *A. nidulans gfdB* gene

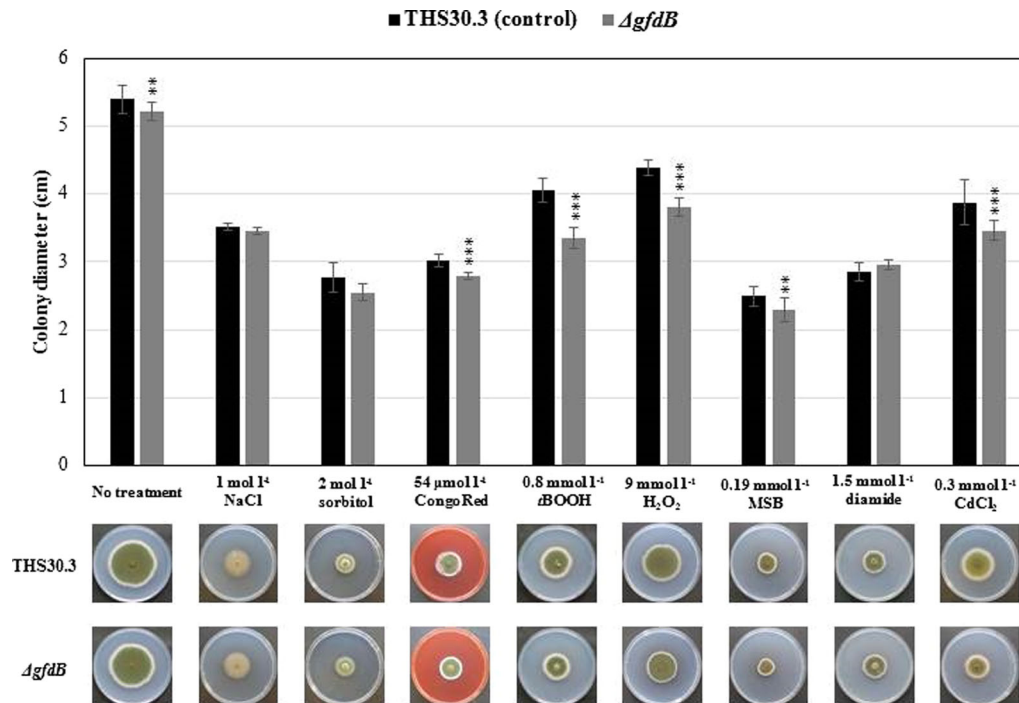


FIGURE 2 Stress tolerances of the THS30.3 (control) and the $\Delta gfdB$ gene deletion mutant *Aspergillus nidulans* strains exposed to various types of stress. Data are presented as mean \pm standard deviation values calculated from three independent experiments. Stress sensitivities observed in surface cultures on nitrate minimal medium agar plates are shown. Plates were incubated at 25°C for 10 days. Significant differences between control and mutant cultures (* $p < .05$, ** $p < .01$, and *** $p < .001$) are indicated

in environmental stress response previously [9], we concluded that the *A. nidulans gfdB* gene kept its stress response-related functions after transferring it to another *Aspergillus* species, *A. glaucus*. In *A. nidulans*, owing to the duplication and the subsequent subfunctionalization/neofunctionalization of the ancient *gfd* glycerol 3-phosphate dehydrogenase encoding gene, *gfdA* and *gfdB* evolved different functions during the evolution. According to the stress sensitivity phenotypes collected with the *A. nidulans* $\Delta gfdA$ and $\Delta gfdB$ gene deletion mutants [8,9], *gfdA* takes part in osmotic stress adaptation meanwhile *gfdB* became an important element of the oxidative stress defense system of the fungus. The *gfdB* gene also strengthens Cd²⁺ stress tolerance, which is understandable because this heavy metal also induces oxidative stress in the Aspergilli [20,23,24]. Importantly, both genes, *gfdA* and *gfdB*, are also involved in the cell wall integrity stress defense of *A. nidulans* [8,9]. It is also noteworthy that the stress sensitivity of the *A. nidulans* $\Delta gfdB$ gene deletion mutant was more moderate at lower incubation temperature (25°C vs. 37°C) most likely owing to better adaptation of the fungus to environmental stress in general at suboptimal growth temperature (at 25°C) [9]. Similarly increased stress tolerances have been recorded for the lactic acid bacterium *Lactobacillus acidophilus* CRL 639 grown at 25°C (vs. 37°C) [25] and maize (*Zea mays* L.) grown at 15°C (vs. 25°C) [26]. In the

baker's yeast *Saccharomyces cerevisiae*, low-temperature cultivations (12–15°C) may increase intracellular reactive oxygen species levels and, consequently, may induce oxidative stress response [27].

In *A. glaucus*, the inserted *gfdB* gene increased the oxidative and cell wall integrity as well as heavy metal stress tolerances but did not affect the osmophilicity of the fungus (Figures 1, S3–S5, and S8–S15 and Table S2). Therefore, we can conclude that the remarkable osmophilicity of *A. glaucus* does not seem to be the consequence of the lack of the *gfdB* gene as we hypothesized before [10].

Unexpectedly, the beneficial effects of *gfdB* supplementation on environmental stress tolerance of *A. glaucus* was only observable in the presence of the nonionic osmolyte sorbitol added at 2.0 mol l⁻¹ concentration. When the culture medium was supplemented with 1.0 mol l⁻¹ NaCl, no similar improvements in the tested stress tolerances were recorded (Figures S12–S15). As demonstrated by the stress physiological data deposited in the Fungal Stress Database (<http://www.fung-stress.org/>; under Growth Profiles) by our research group previously [5,10,12], the addition of 2.0 mol l⁻¹ sorbitol increased the growth and the oxidative (H₂O₂, menadione sodium bisulfite) stress tolerances more than the supplementations with either 0.5 or 1.0 mol l⁻¹ NaCl (25°C, 10 days, NMM). Furthermore, cosupplementation of the

A. glaucus cultures with 2.0 mol l⁻¹ sorbitol and 0.5, 1.0, or 1.5 mol l⁻¹ NaCl decreased the beneficial effects of sorbitol in a dose-dependent manner (5, multimedia component 2), clearly indicating interference between the osmostabilizing effects of the nonionic and ionic osmolytes. Undoubtedly, future studies should aim at the differences in the mechanisms of osmostabilizations by various osmolytes in *A. glaucus* and also their interplays/crosstalks with *gfdB*-dependent environmental stress responses.

The regulatory mechanisms leading to the observed partitions of physiological functions between *gfdA* (contributes to osmotic stress tolerance; [8]) and *gfdB* (involved in oxidative, cell wall integrity, and heavy metal stress defense; [9]; this study) have not yet been elucidated. Nevertheless, they must be quite complex because the expression of *gfdA* did not respond to either osmotic or oxidative stress in *A. nidulans* [13] meanwhile the expression of *gfdB* seems to be osmotic stress-responsive (0.6 mol l⁻¹ NaCl, *A. nidulans*, [13]). Paradoxically, the expression of *gfdB* was not affected by peroxide stresses in either *A. nidulans* (H₂O₂, tBOOH; [10]) or *A. glaucus* (tBOOH; Figure S2) but the NaCl elicited induction of *gfdB* was under AtfA control in *A. nidulans* [10]. Interestingly, the AtfA—a bZIP-type transcription factor is a key player in the orchestration of the oxidative stress response of *A. nidulans* [13,28,29]. Therefore, further studies are also needed to shed light on the molecular background of the interesting osmophilic phenotype of *A. glaucus* and also on the complex regulation of *gfdA* and *gfdB* under various types of environmental stress.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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