Peroxiredoxin (*2-cys-prx*) and catalase (*katA*) cyanobacterial-based bioluminescent bioreporters to detect oxidative stress in the aquatic environment

Peroxiredoxin (*2-cys-prx*) and catalase (*katA*) cyanobacterial-based bioluminescent bioreporters to detect oxidative stress in the aquatic environment

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Abstract

The detection of oxidative stress caused by emerging pollutants in aquatic systems is essential to carry out toxicological analysis since they can bring us information about the mechanisms of toxic action of the pollutants, which might be useful to address this contamination. To achieve this goal, two self-bioluminescent strains that respond to oxidative stress based on the filamentous cyanobacterium *Nostoc* sp. PCC7120, which has a high ecological relevance in aquatic continental systems, have been constructed. *Nostoc* sp. PCC7120 pBG2172 harbours the promoter region of the *2-cys-prx* gene (*P2-cys-prx*), encoding a cytoplasmic peroxiredoxin, fused to *luxCDABE* genes of the bacterium *Photorhabdus luminescens*. *Nostoc* sp. PCC7120 pBG2173 harbours the promoter region of the *KatA* gene (*PkatA*), a cytoplasmic catalase, also fused to *luxCDABE* genes. Both strains have been characterized by exposing them to H_2O_2: *Nostoc* sp. PCC7120 pBG2172 responded while *Nostoc* sp. PCC7120 pBG2173 did not respond to this pollutant. In order to know their specificity, they were exposed to methyl viologen (MV), an herbicide that produces superoxide anion (O_2^-) and a bioluminescence response was observed in both strains. Besides, the utility of these strains for the detection of H_2O_2 and MV in natural water samples, both pristine and wastewater samples has been tested by spiking experiments. Finally, the possible application of these strains for the detection of the emerging pollutant triclosan has also been tested showing to be suitable bioreporters to study oxidative stress in aquatic environments.

Keywords: Cyanobacteria; Bioreporter; Oxidative stress; Catalase; Peroxiredoxin

1. Introduction

Human activity of modern societies has brought with it the deterioration of ecosystems of the entire planet, both aquatic and terrestrial. In aquatic ecosystems, one of the greatest sources of pollution is given by urban, rural and industrial activities, which involve the dumping of large quantities of pollutants to the environment, primarily due to the discharge of contaminants from wastewater systems. The systems to treat wastewater normally are unable to eliminate all co-occurring pollutants (Livingstone, 1998). Furthermore, we do not know the effects of many of these pollutants (emerging pollutants) on the biota. Studies carried out in recent years (Ferreira et al., 2005; Stone and Donaldson, 2006; Lushchak, 2011) have drawn attention on the involvement of oxidative stress as a mechanism of toxicity of many pollutants found in environmental samples.

Oxidative stress appears when an excess in reactive oxygen species (ROS) occurs in the organisms and the antioxidant defences are not capable to eliminate them (Latifi et al., 2009). In the case of photosynthetic organisms such as cyanobacteria, the sources of ROS can be both aerobic respiration and electron transport during photosynthetic activity. Cyanobacteria have been used in a large variety of toxicological studies due to their autotrophic character and their importance in the food chain. Furthermore, cyanobacteria appeared on earth approximately 3000 million of years ago (Schopf, 1993), so they are very well adapted to different kinds of stress including oxidative stress. They possess a large variety of specific enzymes against oxidative stress (Latifi et al., 2009), some important ones are summarized in Table S1. Although superoxide anion ($O_2 \cdot^-$) is one of the most dangerous ROS, its dismutation to H_2O_2 (by superoxide dismutases) is responsible to the production of H_2O_2 (Fridovich, 1997; Li et al., 2002). This ROS is not the most toxic, but it is relativity stable and may form the most dangerous ROS, the hydroxyl radical ($OH\cdot$) via the Fenton reaction (Halliwell and Gutteridge, 1986).

Catalases and peroxiredoxins are the enzymes that remove H_2O_2 converting it into harmless water, so they play an important role in ROS detoxification processes. In *Nostoc* sp. PCC7120, they exist seven peroxiredoxins which remove H_2O_2, alkyl hydroperoxides and peroxynitrites (Dietz, 2011; Cui et al., 2012). These enzymes are divided in typical 2-Cys-Prx peroxiredoxins, atypical 2-Cys-Prx peroxiredoxins (which are subdivided into type II Prx and PrxQ) and 1-

Cys-Prx peroxiredoxins (Wood et al., 2003). The typical 2-Cys-Prx play an important role against oxidative stress in different organisms. In *Nostoc* sp. PCC7120, the gene *alr4641* (*2-cys-prx*) encodes this enzyme which is located in both vegetative cells and heterocyst protecting the cell of oxidative stress by using various reducing agents for detoxifying peroxide (Pascual et al., 2010) (Banerjee et al., 2015). However, to date, only two types of catalases (Mn-catalases) are known in *Nostoc* Sp. PCC7120: KatA (encoded by *alr0998*) and KatB (encoded by *alr3090*). *KatB* is induced by desiccation, iron starvation and in presence of NaCl(Narayan et al., 2011; Katoh, 2012; Chakravarty et al., 2016). Although to date there is scarce information about the catalase activity of KatA in *Nostoc* sp. PCC7120, there are evidence of its protective role against oxidative stress in presence of H_2O_2 (Banerjee et al., 2012).

In contrast with the classical chemical methods to detect contaminants, the use of bioreporters in toxicological studies allows to gather information about the bioavailability and mechanisms of toxic action of the pollutants in the environment. These engineered modified organisms produce a dose-dependent measurable signal in response to chemical or physical agents (Harms et al., 2006; Van Der Meer and Belkin, 2010). Between the most used reporter genes, the bacterial luciferase genes *luxCDABE*, are used to construct self-luminescent bioreporters. Due to their easy maintenance, low cost and their environmentally relevance, cyanobacteria have been used in several works to construct bioreporters (Fernández-Piñas et al., 2014; Hurtado-Gallego et al., 2019). However, to date only two bioreporters that detect oxidative stress have been constructed based on cyanobacteria (Hurtado-Gallego et al., 2018).

In this study, we have constructed two cyanobacterial-based bioreporters which detect $O_2{\cdot}^-$ and H_2O_2 in order to have a battery of cyanobacterial bioreporters capable to detect different ROS in the environment. These strains, based on *Nostoc* PCC7120, were named *Nostoc* PCC7120 pBG2172 and *Nostoc* PCC7120 pBG2173 and harbored the fusion of the promotor region of the *2-cys-prx* and *katA* genes to *luxCDABE* genes, respectively. Both bioreporters were characterized, firstly with H_2O_2 because it is the general substrate for catalases and peroxiredoxins (Table S1). In order to investigate their specificity, they were also characterized with methyl viologen (MV) which induces the production of $O_2{\cdot}^-$ (Lascano et al., 2012). Further validation of the bioreporters was made with the emerging pollutant, triclosan (a wide spectrum biocide) previously described to induce oxidative stress in a diversity of organisms (Binelli et al., 2009; González-Pleiter et al., 2017). Finally, we tested the suitability of the bioreporters to be used in natural matrices in two real waters, one of them a pristine water and the other one a polluted water.

2. Materials and methods

2.1. Chemicals

The chemical material used for the bioluminescence assays were: MV (Aldrich, 98%) prepared at a stock solution of 30 nM in distilled water; H2O2 (Panreac 30%) prepared at stock solution of 2.5 mM in distilled water; triclosan (Sigma) prepared at stock solution of 100 ppm in 70% ethanol.

2.2. Biological material and culture conditions

All the strains used in this work and their culture conditions and characteristics are summarized in Table 1. Briefly, Nostoc sp. PCC7120 strains were cultured in liquid culture medium AA (Allen and Arnon, 1955) (Table S2) diluted 8 times and supplemented with nitrates (5 mM) (Medium AA/8 + N) in continuous orbital shaking at 150 rpm, at constant temperature of 28 °C and light intensity of 65 μmol of photons m2/s. When the strains were grown on solid medium, undiluted AA medium was used, supplemented with nitrates (5 Mm) and 1% w/v of purified agar.

Escherichia coli (*E. coli*) strains (Table 1), were cultured in liquid medium LB (Luria-Bertani) composed of yeast extract 0.5% w/v, bacteriotriptone 1 % w/v and NaCl 0.5 % w/v, incubated at a constant temperature of 37 °C and agitation of 250 rpm. LB medium was also used for the growth in solid medium, to which bacteriological agar 1 % w/v was added and were incubated at 37 °C without agitation.

2.3. Construction of bioreporter strains

The construction of cyanobacterial bioreporters were carried out such as previously reported (Hurtado-Gallego et al., 2018). Briefly, primers for the amplification of the promoter regions of *alr4641* (*2-cys-prx*) and *alr0998* (*katA*) genes were designed. Primers sequences and size of PCR amplification products are summarized in Table S3. These primers amplify these promoter regions and the restriction sites for the *Kpn*I/*Sal*I enzymes. The amplification products were cloned into the plasmid pBG2146 (Hurtado-Gallego et al., 2018) in the *Kpn*I/*Sal*I sites, generating the plasmids: pBG2172 (Fig. 1A), harbouring the *2-cys-prx* promoter and pBG2173 (Fig. 1C), harbouring the *katA* promoter. Parental plasmid pBG2146 bears the T4 transcriptional terminator in order to ensure minimum baseline luminescence. The resulting plasmids pBG2172 and pBG2173 contain a *P2-cys-prx*::*luxCDABE* and *PkatA*::*luxCDABE* transcriptional fusion, respectively. Restriction analysis and DNA sequencing (data not shown) confirmed the integrity of the constructions in *E. coli*. Each plasmid was introduced into *Nostoc* sp. PCC7120 wild type by triparental conjugation as previously described (Elhai and Wolk, 1988; Elhai et al., 1997). The integrity of the transformation in *Nostoc* sp. PCC7120 wild type was confirmed by PCR (Fig. S1). The nucleotide sequences

Table 1. Bacterial strains used in this study.

Strain	Plasmid	Characteristics
Nostoc sp. PCC7120	–	Wild type strain from which the bioreporter strains were constructed
N. sp. PCC7120 pBG2146	pBG2146. Parental vector which harbours the T4 transcriptional terminator and the *luxCDABE* genes	Bioreporter strain of *Nostoc* sp. PCC7120 expressing the plasmid pBG2146. SpR in AA/8 + N growth medium
N. sp. PCC7120 pBG2172	pBG2172. Plasmid derived from pBG2146 that harbours the promoter region of the *2-cys-prx* gene fused to the *luxCDABE* genes	Bioreporter strain of *Nostoc* sp. PCC7120 expressing the plasmid pBG2172. SpR in AA/8 + N growth medium
N. sp. PCC7120 pBG2173	pBG2173. Plasmid derived from pBG2146 that harbours the promoter region of the katA gene fused to the luxCDABE genes	Bioreporter strain of *Nostoc* sp. PCC7120 expressing the plasmid pBG2173. SpR in AA/8 + N growth medium
Escherichia coli DH5α pBG2172	pBG2172 (see above)	Competent *E. coli* DH5α strain that has been transformed to harbour the plasmid pBG2172. SpR in LB growth medium
E. coli DH5α pBG2173	pBG2173 (see above)	Competent *E. coli* DH5α strain that has been transformed to harbour the plasmid pBG2173. SpR in LB growth medium
E. coli pRL623	pRL623. Conjugative plasmid which harbours the genes that encode the AvaI/II/III methylases and the *mob* gene that generates a cut in the oriT region of the plasmids pBG2172 and pBG2173 for the conjugation	*E. coli* competent strain harbouring the plasmid pRL623. Used in triparental conjugation. Cm 25R in LB growth medium
E. coli pRL623/pRL2172	*E. coli* strain used in triparental conjugation harbouring the plasmids pRL623 and pBG2172	Cm^R and Sp^R in LB growth medium
E. coli pRL623/pRL2173	*E. coli* strain used in triparental conjugation harbouring the plasmids pRL623 and pBG2173	Cm^R and Sp^R in LB growth medium
E. coli pRK2013	pRK2013. Conjugative plasmid derived from RP4 for the expression of proteins that form the pilus necessary for conjugation	*E. coli* strain used in conjugation. Km^R in LB growth medium

of *2-cys-prx* and *katA* promoter regions used in the fusions are depicted in Fig. 1; by bioinformatic analysis (using BPROM tools at Softberry, http://www.softberry.com/) The −10 and −35-like identified promoter regions for both ORFs are indicated in Fig. 1B and D, respectively. The ribosome binding sites (SD; (Shine and Dalgarno, 1975)), and the translational start codons (ATG) are also denoted (boxed).

2.4. Bioluminescent induction bioassays for the characterization of the bioreporters

To check how the bioreporter strains *Nostoc* sp. PCC7120 pBG2172 and *Nostoc* sp. PCC7120 pBG2173 responded to oxidative stress, different pollutants were used: H_2O_2 (in a range of 0–100 μM) and MV (in a range of 20–5000 pM). A further validation was performed with the biocide triclosan (in a range of 0–0.051 mM). As a control, strain *Nostoc* sp. PCC7120 carrying pBG2146 (parental vector with no transcriptional fusion, see Fig. 1) was also exposed to the different pollutants.

These characterization experiments were carried out as previously described (Hurtado-Gallego et al., 2018). Briefly, before the exposure to the compounds, the cells were grown until their mid-log phase. Then, in order to standardize the experiments, the cells were resuspended in fresh AA/8 + N medium (without antibiotic) at a final $OD_{750nm} = 0.5$ (Rodea-Palomares et al., 2009). The characterization of the bioreporters consisted in the exposure of the strains to increasing concentrations of the compounds up to 24 h in transparent 24 well microplates in a final volume of 1.5 mL.

The plates were incubated at 28 °C at 150 rpm and 65 μmol of photons m^2/s of light intensity. Aliquots of 100 μl were taken in duplicate at specified times up to 24 h of exposure from each well and were transferred to

96-well white plates and taken to be measured in a Berthold Centro LB960 luminometer, taking a total of nine luminescent measurements for 20 min.

The data were calculated dividing the bioluminescence mean of the treated sample by the bioluminescence mean of the unexposed cells (control) and were expressed as Bioluminescence Induction Factor (BIF). The lowest pollutant concentrations with significant differences with respect to the untreated samples were considered as the Limit Of Detection (LODs). At longer times of exposure and/or higher concentrations of the pollutant, toxicity was evident due to the decrease in bioluminescence; thus, the highest pollutants concentrations, which did not cause toxicity to the bioreporters, were defined as the Maximum Permissible Concentrations (MPCs) (see Statistical Analysis).

2.5. Spiking bioassays in natural waters

In order to evaluate the response of both bioreporters in natural matrices two types of water were used. The first sample of water comes from near the head waters of the Guadalix River (Glx1) (Douterelo et al., 2004), as an example of pristine water not contaminated and previously characterized (Fernández-Piñas et al., 2014). The other sample of water that was used comes from the effluent of the Alcalá de Henares wastewater treatment plant and it is a water with a high concentration of nutrients, organic matter and other types of emerging contaminants also previously characterized (Rosal et al., 2010b; Gómez et al., 2012). The basic physicochemical characteristics of the two types of water used in this study are shown in Table S4.

To carry out the spiking bioassays, the bioreporters cultures were processed as previously described in section 2.4 "Bioluminescent induction bioassays for the characterization of the bioreporters", except for the last resuspension of the cells which, instead of in AA/8 + N medium, were resuspended in the water samples. The concentrations of H_2O_2 used in the spiking experiments were from 12 to 40 μM. The MV concentrations used were from 500 to 1000 pM. In parallel, a control was performed in AA/8 + N medium, whose purpose was to verify that the natural water itself did not induce bioluminescence in any bioreporter. They were incubated at 28 °C at 150 rpm of agitation and 65 μmol of photons m^2/s of light intensity for 1.5 h and 6 h in *Nostoc* sp. PCC7120 pBG2172 after the exposure to H_2O_2 and MV respectively. In *Nostoc* sp. PCC7120 pBG2173 this induction was carried out during 3 h.

2.6. Statistical analysis

A minimum of three independent experiments were performed with three replicates for each assay situation. All test of statistically significant differences between data sets were performed using Student's *t*-test and one-way analysis of variance (ANOVA) which were computed using R analysis package (R for windows, 3.0.2 copyright© The Foundation for Statistical Computing).

3. Results

3.1. Characterization of the bioluminescence response of *Nostoc* sp. PCC7120 pBG2172 and *Nostoc* sp. PCC7120 pBG2173

To check how the bioreporters *Nostoc* sp. PCC7120 pBG2172 and *Nostoc* sp. PCC7120 pBG2173 responded to different ROS, the model pollutants H_2O_2 and MV (as O_2^- producer) (Lascano et al., 2012) were used. Table 2 summarized the LODs, MPCs and the main parameters of the regression curves of both bioreporters response to H_2O_2 and MV.

The induction profile of *Nostoc* sp. PCC7120 pBG2172 after the exposure to H_2O_2 in a concentration range from 0 to 150 μM is shown in Fig. 2A. These profiles show the bioluminescence up to 6 h of exposure, since no further bioluminescence induction was observed at any concentration at longer times. The values of bioluminescence induction factor (BIF) varied depending on the H_2O_2 concentration and the exposure time, following a dose-response curve between 0.5 and 3 h. After 3 h of exposure, the toxicity produced by the chemical was noticeable in the highest concentration since the bioluminescence values decreased and the differences observed were no longer statistically significant (ANOVA $p < 0.05$) (Fig. 2A). The detection range of the strain *Nostoc* sp. PCC7120 pBG2172 was 10–100 μM of H_2O_2, since it was the concentrations range at which a statistically significant induction of

Table 2. Main parameters of bioluminescence response of both bioreporters to H_2O_2 and MV. Limits of detection (LODs), maximum permissive concentrations (MPCs), maximum linear range, regression equations and corresponding R^2 values.

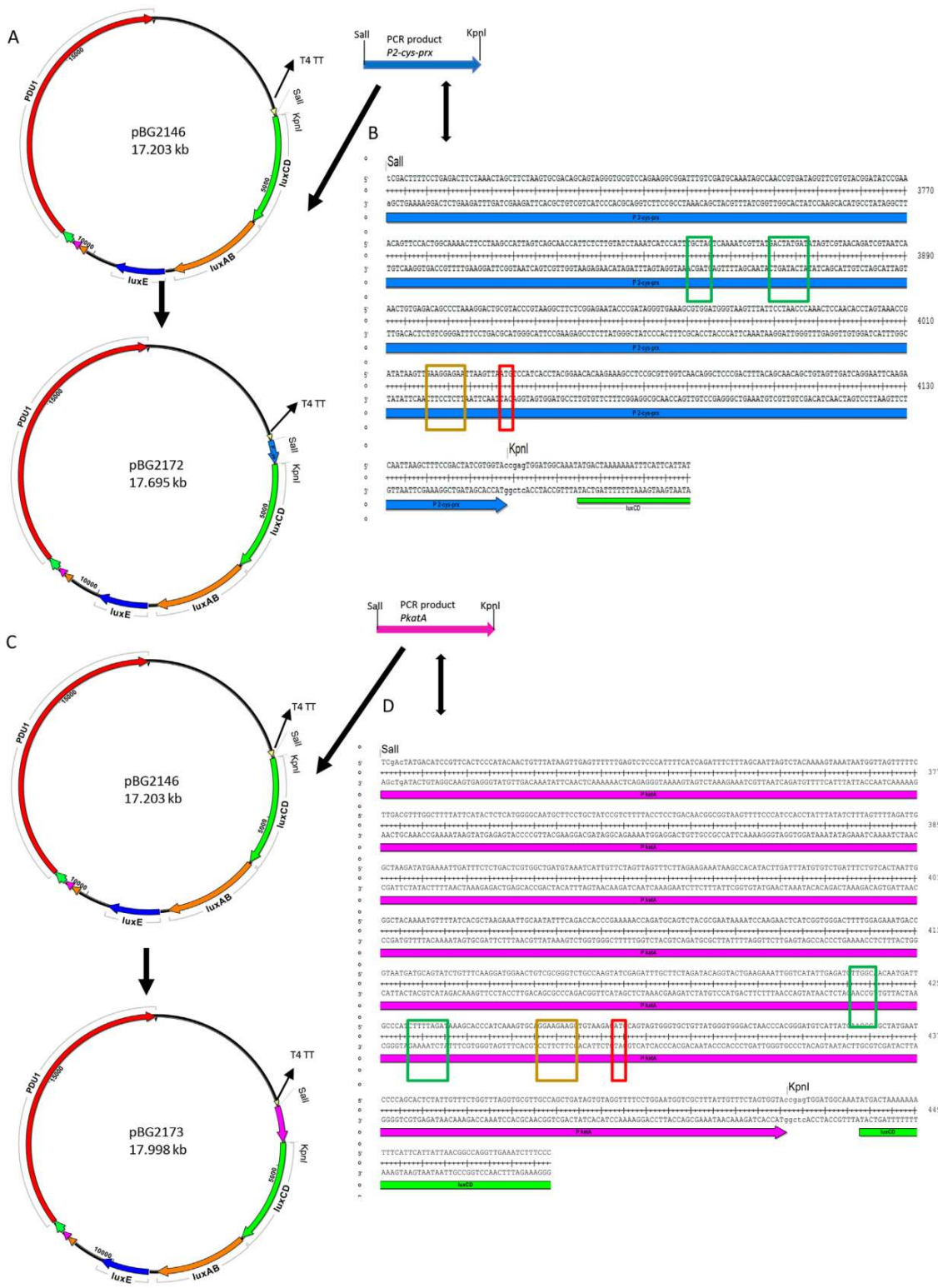|	LODs		MCPs		Maximum linear range		Regression equation		R^2	
	H_2O_2 (mM)	MV (pM)	H_2O_2 (mM)	MV (pM)	H_2O_2 (mM)	MV (pM)	H_2O_2 (mM)	MV (pM)	H_2O_2 (mM)	MV (pM)
Nostoc sp. PCC7120 pBG2172	10	50	100	2500	10-50	50-800	y = 0.052x + 5.40	y = 0.009x + 3.42	0.97	0.93
Nostoc sp. PCC7120 pBG2173	-	200	-	1000	-	200-1000	-	y = 0.003x + 1.03	-	0.98

Figure 1. Strategy of construction of the plasmids pBG2172 and pBG2173 and sequence of the promoter regions of *2-cys-prx* and *katA* genes used in the transcriptional fusions. A) Construction of the pBG2172 plasmid via fusion of the PCR product containing the promoter region of gene *2-cys-prx* cut with *Sal*I and *Kpn*I from genomic DNA and inserted into the same restriction sites of replicating plasmid pBG2146 upstream of *luxCDABE* genes. T4TT: T4 transcriptional terminator. B) The nucleotide sequence of *2-cys-prx* promoter region used in the fusion is depicted; the predicted sequence corresponding to the −10 and −35 regions of the *2-cys-prx* promoter (boxed in green), the ribosome binding site (SD) (boxed in orange), and the translational start codon (ATG) (boxed in red) are denoted. The initial sequence of *luxCDABE* is underlined in green. C) Construction of the pBG2173 plasmid via fusion of the PCR product containing the promoter region of gene *katA* cut with *Sal*I and *Kpn*I from genomic DNA and inserted into the same restriction sites of replicating plasmid pBG2146 upstream of *luxCDABE* genes. T4TT: T4 transcriptional terminator D) The nucleotide sequence of *katA* promoter region used in the fusion is depicted; the predicted sequence corresponding to the −10 and −35 regions of the *katA* promoter (boxed in green), the ribosome binding site (SD) (boxed in orange), and the translational start codon (ATG) (boxed in red) are denoted. The initial sequence of *luxCDABE* is underlined in green.

bioluminescence was observed with respect to the control, so the MPC of H_2O_2 for *Nostoc* sp. PCC7120 pBG2172 was 100 µM (Table 2). The limit of detection (LOD) observed was 10 µM of H_2O_2, since no induction of the luminescence was observed at lower concentrations. The maximum induction peak occurred after 1.5 h to 50 µM of H_2O_2, which generated a BIF close to eight-fold (the bioluminescence induced by this

H_2O_2 concentration was 8 times higher than the basal bioluminescence of the strain). Above this concentration the bioluminescence began to decrease probably due to the toxic effect of the compound. An exposure time of 1.5 h was used to make a regression equation (10–50 µM) (Table 2). No bioluminescence induction was observed after the exposure to H_2O_2 in *Nostoc* sp. PCC7120 pBG2173 (Fig. 2B).
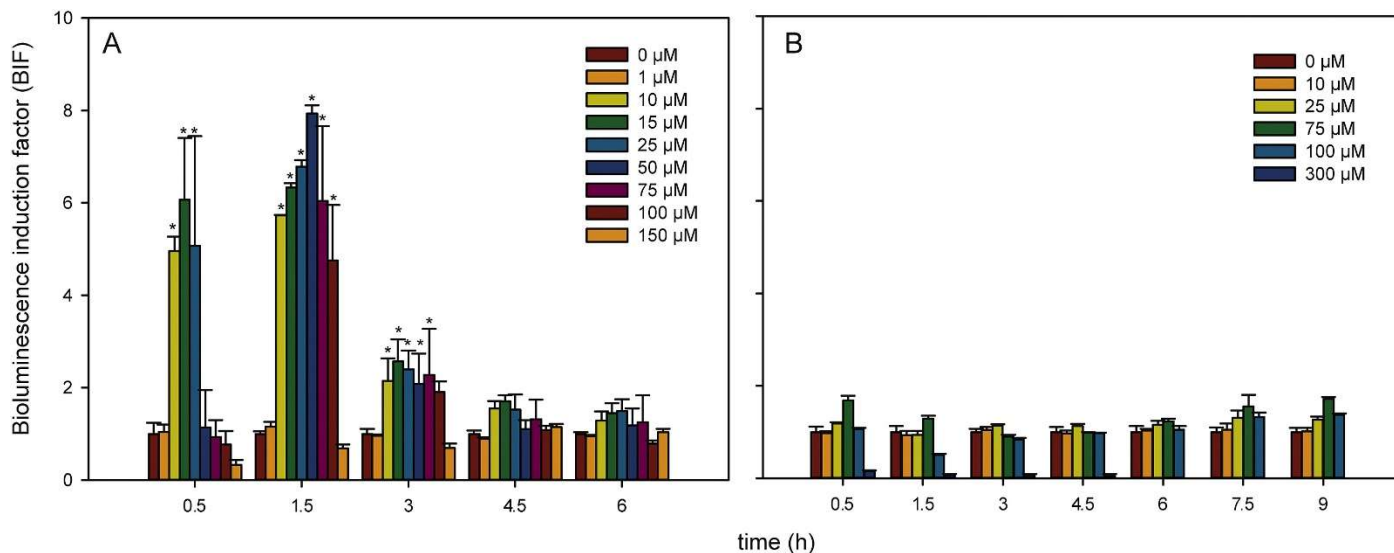

Figure 2. Bioluminescent response of both cyanobacterial bioreporters after the exposure to H_2O_2. A) represents the response of *Nostoc* PCC7120 pBG2172 after 6 h of exposure to H2O2, B) represents the response of *Nostoc* PCC7120 pBG2173 after 9 h of exposure to H_2O_2. The asterisks indicate the statistically significant differences with respect to the control (unexposed cells) (ANOVA p < 0.05).

In order to study their ROS specificity, the bioreporters were also exposed to MV, which induces the formation of another ROS, $O_2\cdot^-$. In this case, both bioreporters showed bioluminescence induction. Fig. 3A shows the induction profile during 12 h of *Nostoc* sp. PCC7120 pBG2172 after the exposure to 0–3500 pM of MV. From this time on, the differences observed were not statistically significant (ANOVA $p < 0.05$) (data not shown). As in the H_2O_2 case, the BIF values varied depending on the concentration of MV used and the exposure times, following a dose-response curve between 1.5 and 12 h. After 1.5 h of exposure, the luminescence maximum was achieved at 1200 pM, with a BIF value of approximately twelve-fold. After this time, the induction of the luminescence remained practically stable until 6 h of exposure, after which it began to decrease progressively and disappeared after 12 h of exposure (data not shown) where the differences observed were not statistically significant (ANOVA $p < 0.05$). As *Nostoc* sp. PCC7120 pBG2172 was able to respond to MV following a dose-response curve, it can be concluded that the strain was not specific for H_2O_2, since it was responding to $O_2\cdot^-$ produced by the MV. Although the detection range was 50–2500 pM of MV after 1.5 h of exposure, the regression equation was calculated after 6 h of exposure to MV as at this time of exposure, the maximum linear range of bioluminescent response (50–800 pM) (Table 2) was observed.

In the *Nostoc* sp. PCC7120 pBG2173 case, the bioluminescence also responded to MV following a dose-response curve at same times of exposure (Fig. 3B). The maximum bioluminescence response was observed after 7.5 h of exposure to 500 pM of MV and was around ten-fold with respect to the control (unexposed cells). After this time, de bioluminescence decreased due to the toxic effect of MV to the cells. The LOD was 200 pM of MV and the MPC was 1000 pM (Table 2). The detection range and the maximum linear range after these times of exposure were the same (200–1000 pM). With these data, the regression equation was calculated.

Both bioreporters presented very different bioluminescence profiles, denoting the differences between the genes *2-cys-prx* and *katA*. While *Nostoc* sp. PCC7120 pBG2172 showed bioluminescence to both ROS (H_2O_2 and $O_2\cdot^-$), *Nostoc* sp. PCC7120 pBG2173 showed a specific response to only $O_2\cdot^-$.

No bioluminescence induction was observed after the exposure to H_2O_2 or MV of control strain *Nostoc* sp. PCC7120 carrying parental vector pBG2146 (Fig. S2 A, B), indicating that the observed bioluminescence induction in *Nostoc* sp. PCC7120 pBG2172 and *Nostoc* sp. PCC7120 pBG2173 exposed to the pollutants truly corresponded to the response of the used promoters.
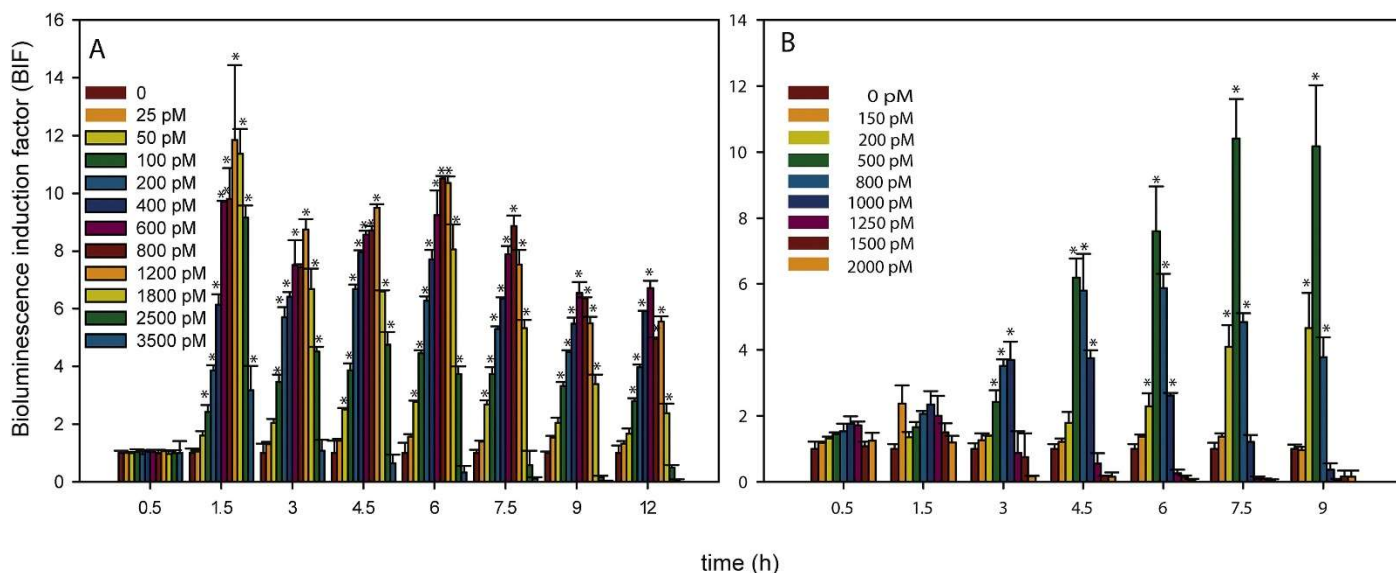
Figure 3. Bioluminescent response of both cyanobacterial bioreporters after the exposure to MV. A) represents the response of *Nostoc* PCC 120 pBG2172 after 12 h of exposure to MV, B) represents the response of *Nostoc* PCC7120 pBG2173 after 9 h of exposure to MV. The asterisks indicate the statistically significant differences with respect to the control (untreated cells) (ANOVA p < 0.05).x

3.2. Application of *Nostoc* sp. PCC7120 pBG2172 and *Nostoc* sp. PCC7120 pBG2173 to detect the ROS produced by the emerging pollutant triclosan

After this characterization of the bioreporters, they were tested for their possible application for detecting ROS produced by emerging pollutants. We used the biocide triclosan, due to its environmental importance and because its mode of action has been previously studied and it is based on the generation of ROS, especially O_2^- (Binelli et al., 2009).

The induction profiles of the *Nostoc* sp. PCC7120 pBG2172 and *Nostoc* sp. PCC7120 pBG2173 for a range of triclosan concentrations from 0 mM to 0.051 mM are shown in Fig. 4. The induction profiles are shown for 6 h of exposure in the case of *Nostoc* sp. PCC7120 pBG2172 and for 9 h of exposure in the *Nostoc* sp. PCC71220 pBG2173 case, since no statistically significant results were observed at longer times (data not shown).

As shown in Fig. 4, the bioluminescence profiles were quite different. *Nostoc* sp. PCC7120 pBG2172 responded significantly to the increasing concentrations of triclosan, showing a typical dose-response curve between 0.5 and 3 h of exposure, after which the differences were not statistically significant (ANOVA $p < 0.05$). The highest induction was found after 1.5 h of exposure, in which the concentration 0.025 mM generated an induction of more than four-fold with respect to the control. Above this time, the induction dropped due to the toxic effect of triclosan in the strain (Fig. 4A). *Nostoc* PCC7120 pBG2173 also showed a dose-response curve but between 3 and 9 h of exposure, after which the differences were not statistically significant (ANOVA $p < 0.05$). The maximum

induction was also after the exposure to 0.025 mM of triclosan but after 7.5 h of exposure reaching around twelve-fold bioluminescence with respect to the control (unexposed cells). After this time, the bioluminescence induction began to drop due to the toxic effect of triclosan.

The LOD for triclosan was similar in both bioreporters. However, *Nostoc* sp. PCC7120 pBG2172 was able to detect higher concentrations of triclosan and in a shorter time than *Nostoc* sp. PCC7120 pBG2173 whose bioluminescence induction was higher (Fig. 4). Despite their differences, both strains were capable of detecting the ROS produced by triclosan, so they may be suitable to detect the ROS produced by emerging pollutants.

No bioluminescence induction was observed after the exposure to triclosan of control strain *Nostoc* sp.PCC7120 carrying parental vector pBG2146 (Fig. S2 C), indicating that the observed bioluminescence induction in *Nostoc* sp. PCC7120 pBG2172 and *Nostoc* sp. PCC7120 pBG2173 exposed to the pollutant truly corresponded to the response of the used promoters.

3.3. Evaluation of the response of *Nostoc* sp. PCC7120 pBG2172 and *Nostoc* sp. PCC7120 pBG2173 to detect ROS in natural waters: spiking experiments

Once the response of the cyanobacterial strains in AA/8 + N growth medium was characterized, the possible application of these organisms for the detection of H_2O_2 and MV in natural waters was evaluated. Spiking experiments were carried out, following the methodology described in "2.5 Spiking bioassays in natural waters" section. For this, two samples of natural waters were used: a sample of water from near the head waters of the Guadalix River (Glx1) as an example of
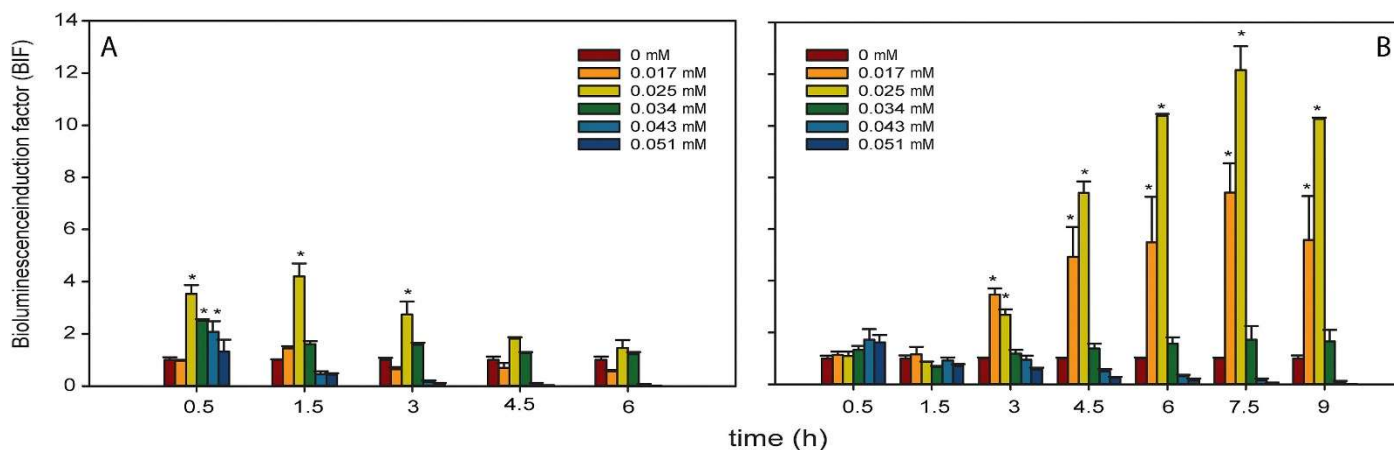
Figure 4. Bioluminescent response of both cyanobacterial bioreporters after the exposure to triclosan. A) represents the response of *Nostoc* PCC7120 pBG2172 after 6 h of exposure to triclosan; B) represents the response of *Nostoc* PCC7120 pBG2173 after 9 h of exposure to triclosan. The asterisks indicate the statistically significant differences with respect to the control (untreated cells) (ANOVA $p < 0.05$).

pristine water (Muñoz-Martín et al., 2014), and a sample of water obtained from the effluent from the Alcalá de Henares waste-water treatment plant (Rosal et al., 2010b) and (Gómez et al., 2012) as an example of water containing diverse pollutants. Previously to the spiking experiments, the bioreporters were exposed to the natural waters and no bioluminescence induction was observed (which was indicative that none of the compounds present in the waters or the physicochemical characteristics of both waters cause oxidative stress in the strains). For the spiking experiments, the matrices were artificially polluted with H_2O_2 or MV concentrations. Table 3 shows the nominal H_2O_2 and MV added concentrations used in spiking experiments and the bioavailable concentrations detected by the bioreporters. This bioavailable concentration was estimated from the regression equations of each bioreporter, in the case of *Nostoc* sp. PCC7120 pBG2173 only for MV as no bioluminescence was observed after the exposure to H_2O_2 (Fig. 2B).

Nostoc sp. PCC7120 pBG2172 was able to detect both H_2O_2 and MV in both water samples. The bioavailable concentration of the compounds calculated was within the order of magnitude of the nominal concentrations used in all cases, except for MV in WWTP, that was lower than the nominal concentration. This phenomenon was also observed in *Nostoc* sp. PCC7120 pBG2173, where a higher MV concentration was used in order to detect it (Table 3). This response to MV of the bioreporters has been previously observed in cyanobacterial bioreporters (Hurtado-Gallego et al., 2018), due to the formation of complexes between the MV and the organic matter and co-occurring contaminants present in this water sample, which would reduce the bioavailability of the pollutant.

Despite the differences existing according to the matrix used, it was verified that the bioreporter strains *Nostoc*

sp. PCC7120 pBG2172 and *Nostoc* sp. PCC7120 pBG2173 are valid to detect the compounds used in this study in natural matrices of different characteristics.

4. Discussion

In this work we have constructed two bioluminescent strains based on *Nostoc* sp. PCC7120, denoted as *Nostoc* sp. PCC7120 pBG2172 (*P2-cys-prx::luxCDABE*) and *Nostoc* sp. PCC7120 pBG2173 (*PkatA::luxCDABE*). Both of them have been characterized in response to oxidative stress caused by H_2O_2 and MV (which generates O_2^-) and their applicability in natural waters has been evaluated as well as their capacity to detect emerging contaminants such as triclosan. As can be seen in Table 4, the bacterium most widely used to date for the construction of bioreporter that detect oxidative stress in aquatic environments has been *E. coli*. The regulation system of *E. coli* is based on OxyR and SoxRS, induced by O_2^- and H_2O_2 (Robbens et al., 2010). The main advantage of using cyanobacteria for the construction of this type of bioreporter is their ecological relevance. Cyanobacteria are autotrophic organisms, which confers them the role of primary producers of the trophic chain in aquatic ecosystems, so that any alteration in their populations will directly affect higher trophic links, producing adverse effects in them. In addition, cyanobacteria are considered as the first organisms responsible for emitting oxygen into the atmosphere through oxygenic photosynthesis, which could explain the wide variety of molecular systems based on proteins involved in detoxifying the ROS to cope with the oxidative stress (Latifi et al., 2009).

As shown in Fig. 2A, the LOD of the strain *Nostoc* sp. PCC7120 pBG2172 after the exposure to H_2O_2 was 10 μM and the detection range was 10–100 μM after 1.5 h of exposure. Comparing these data with the rest of bacterial-based bioreporters that detect ROS (Table 4), it can be observed that the cyanobacterial-strain is the

Table 3. Concentrations of nominal and bioavailable H_2O_2 and MV calculated form the regression equation of *Nostoc* sp. PCC7120 pBG2172 and *Nostoc* sp. PCC7120 pBG2173 (Table 2) in natural water samples (Glx1 and WWTP). The times of exposure varied depending on the already characterized bioreporter response and the pollutant: *Nostoc* sp. PCC7120 pBG2172 was exposed 1.5 h to H_2O_2 and 6 h to MV. *Nostoc* sp. PCC7120 pBG2173 was exposed 3 h to MV

	Water sample	H_2O_2 nominal concentration (mM)	Bioavailable H_2O_2 (mM)	MV nominal concentration (pM)	MV nominal concentration (pM)
Nostoc sp. PCC7120 pBG2172	Glx1	18	11.3 ± 13.7	500	574.7 ± 84
		30	16.4 ± 9.6	650	786.3 ± 110
		40	39.4 ± 10.8	750	860 ± 105
	WWTP	18	-	1000	938.9 ± 52.7
		30	18.1 ± 9.7	500	-
		40	18.1 ± 9.7	650	125 ± 102.8
Nostoc sp. PCC7120 pBG2173	Glx1	-	-	750	244.2 ± 144.7
				1000	412.6 ± 105.3
				300	195.9 ± 19.95
				700	472.4 ± 38.20
				1000	656.7 ± 109.9
	WWTP	-	-	300	-
				700	-
				1000	-
				5000	-

most sensitive bacterial bioreporter constructed to date to H_2O_2. For example, *E. coli* DPD2511 (Belkin et al., 1996) which harbours the promoter of *katG* gene, has a ten-fold higher LOD than this cyanobacterial bioreporter after the exposure to H_2O_2. In the case of the *E. coli* strain constructed by Lee and Gu (2003), its LOD to H_2O_2 is 100 times higher than that of *Nostoc* sp. PCC7120 pBG2172. The strain EBHJ constructed by Lee and Gu (2003) presents a detection range between 1000 and 50000 μM of H_2O_2, concentrations that are completely toxic and lethal for cyanobacteria. Banerjee et al. (2012) (Banerjee et al., 2012), generated a recombinant cyanobacterial strain which overexpressed *katA* gene, and demonstrated that this gene responded to oxidative stress caused by H_2O_2. Despite this high sensitivity of cyanobacterial bioreporter, the *E. coli* strains constructed can provide complementary information about the contamination of an environment, since they have greater tolerance of exposure to higher concentrations of H_2O_2.

H_2O_2 is a relatively stable ROS that is naturally present in water bodies by photochemical reactions mediated by natural organic matter, by input from rainfall or by metabolic reactions of many microorganisms. It is dangerous mostly by the fact that it can permeate into cells where it can directly inactivate enzymes and, by Fenton reaction, it may produce the most destructive radical, the hydroxyl radical ($OH^.$) which may damage any macromolecule in its vicinity. Hydrogen peroxide has been measured at concentrations ranging between 10^{-7} and 10^{-11} M in natural surface waters (Cooper and Zika, 1983; Szymczak and Waite, 1988, 1991; Moore et al., 1993; Peake and Mosley, 2004), being normally in the micromolar range in atmospheric water (Kok et al., 1978; Zika et al., 1982). Concentrations in surface waters are generally highest at the photic zone and

decreases towards the detection limit of most methods in the dark waters below the mixed surface layer, reflecting its dominant formation process by photochemical reactions (Burns et al., 2012). In soil, H_2O_2 generated by fungi and bacteria can reach concentrations in the range 2–15 μM in the surrounding microenvironment (Cooper and Lean, 1989; Gonzalez-Flecha and Demple, 1997; Watts et al., 2007). Regarding heavily contaminated environments, H_2O_2 concentrations in the range 1–1000 μM have been measured in groundwater adjacent to spent nuclear fuel waste by the effect of radiation in water (Amme et al., 2005, 2012). Then, it should be taken into consideration that in many contaminated environments, Fe species might be present and the production of the highly dangerous hydroxyl radical from hydrogen peroxide might be of concern.

However, the strain *Nostoc* sp. PCC7120 pBG2173, which harboured *PkatA* did not show any bioluminescence response after the exposure to H_2O_2 (Fig. 2B). In their study (Banerjee et al., 2012) *katA* overexpressing cells survived more than control cells after 2 days of exposure to H_2O_2 (1 mM). However, in the wild type cells (without *katA* overexpression) after the exposure to 1 mM of H_2O_2, peroxiredoxin 2-Cys-Prx was increased, but not KatA (Banerjee et al., 2012). Besides, by Northern blot analysis, (Banerjee et al., 2012) did not find any induction of this gene when *Nostoc* (*Anabaena*) sp. PCC7120 was treated with H_2O_2 or MV at relatively high concentrations. The FurA protein in *Nostoc* sp. PCC7120 is a global regulator and it is involved in oxidative stress protection, in fact it has been found to be a negative regulator of *alr*4641 (2-Cys-Prx) (González et al., 2011; Fillat, 2014). Furthermore, in mycobacteria it has been reported that FurA (a second regulator of the oxidative stress

Table 4. Oxidative stress bioreporters based on bacteria.

Strain	Gene	Specificity	LOD	Range of detection	Reference
Escherichia coli DPD2515	*micF*	H_2O_2	-	-	(Belkin et al., 1996)
		$O_2^{.-}$	4×10^4 pM MV		
E. coli DPD2511	*katG*	H_2O_2	100 µM H_2O_2	-	(Belkin et al., 1996)
		$O_2^{.-}$	2×10^7 pM MV		
E. coli PGRFM	*pgi*	H_2O_2	-	-	(Niazi et al., 2008)
		$O_2^{.-}$	2.4×10^6 pM MV	$2.4 \times 10^6 - 2.4 \times 10^9$ pM MV	
E. coli EBHJ	*sodA*	H_2O_2	1000 µM H_2O_2	1000-50000 µM H_2O_2	(Lee and Gu, 2003)
		$O_2^{.-}$	6×10^4 pM MV	$6 \times 10^4 - 6 \times 10^7$ pM MV	
E. coli ZWF RFM443	*zwf*	$O_2^{.-}$	9.7×10^9 pM MV	-	(Niazi et al., 2007)
E. coli ZWF RFM443	*fpr*	$O_2^{.-}$	7.7×10^{10} pM MV	-	(Niazi et al., 2007)
E. coli K12	*sodA*	$O_2^{.-}$	3.8×10^4 pM MV	$3.8 \times 10^4 - 3.8 \times 10^8$ pM MV	(Ivask et al., 2010)
[a] *E. coli* DK1	*katG*	H_2O_2	-	-	(Hwang et al., 2008)
[a] *E. coli* DS1	*sodA*	$O_2^{.-}$	-	-	(Hwang et al., 2008)
Pseudomonas fluorescens pUCD607	*pUCD607*	$O_2^{.-}$	7.7×10^7 pM MV	$7.7 \times 10^7 - 7.7 \times 10^9$ pM MV	(Porteus et al., 2000)
Nostoc PCC7120 pBG2154	*sodA*	$O_2^{.-}$	400 pM MV	400-1000 pM MV	(Hurtado-Gallego et al., 2018)
N. PCC7120 pBG2165	*sodB*	$O_2^{.-}$	500 pM MV	500-1800 pM MV	(Hurtado-Gallego et al., 2018)
N. PCC7120 pBG2172	*2-cys-prx*	H_2O_2	10 µM H_2O_2	10-100 pM MV	This study
		$O_2^{.-}$	50 pM MV	50-2500 pM MV	
N. PCC7120 pBG2173	*katA*	$O_2^{.-}$	200 pM MV	200-1000 pM MV	This study

[a] These bioreporters were tested with silver nanoparticles.

response) negatively controls *katG* (Zahrt et al., 2001). With this knowledge and the results, it can be said that under normal conditions (without *katA* overexpression), the H_2O_2 detoxification in *Nostoc* sp. PCC7120 is carried out by other enzymes such as peroxiredoxins like 2-Cys-Prx but apparently not by KatA. However, this gene is involved in oxidative stress protection as it responds to $O_2^{.-}$. The fact that *katA* is not directly induced by its substrate (H_2O_2) is puzzling and more studies about regulation of this gene are needed.

On the other hand, the exposure of the strains to MV allowed the characterization of their response to $O_2^{.-}$ in order to check their ROS specificity. As can be seen in the results (Fig. 3), the LOD of the strain *Nostoc* sp. PCC7120 pBG2172 for MV was 50 pM and the detection range was between 50 pM and 2500 pM. For the strain *Nostoc* sp. PCC7120 pBG2173, the LOD was 200 pM and the detection range was between 200 and 1000 pM after the exposure to MV. There are a lot of studies that use MV for bioreporter characterization in response to oxidative stress particularly *E. coli* bioreporters (Table 4). As can be seen in Table 4, *Nostoc* sp bioreporters have the lowest LODs of all the strains after the exposure of MV. Furthermore, within cyanobacteria bioreporters, strain *Nostoc* sp. PCC7120 pBG2172 has the lowest LOD of MV, converting it in the most sensitive strain to MV ever constructed.

It is remarkable that the use of a cyanobacterium such as *Nostoc* sp. PCC7120, in addition to the greater ecological importance previously commented, supposes an enormous increase of the sensitivity to ROS. Nevertheless, the rest of strains constructed are very important to complement the information provided by cyanobacterial bioreporters, since they respond to higher concentrations of MV. As can be observed in Table 4, bioreporters based on *E. coli* or *P. fluorescens* are able to tolerate MV concentrations up to 3.8×10^8 pM and 7.7×10^9 pM, as is the case of the strain *E. coli* K12 constructed by Ivask and Cabbage. (2010) (Blinova et al., 2010) and *P. fluorescens* strain pUCD607 constructed by (Porteus et al., 2000), respectively.

A notable feature to highlight of the strain *Nostoc* sp. PCC7120 pBG2172 is that it is a strain that can respond to both H_2O_2 and $O_2^{.-}$; thus, it is a non-specific oxidative stress bioreporter. Although peroxiredoxins are commonly studied as proteins belonging to the signalling pathways for peroxides such as H_2O_2 (Hall et al., 2009), their induction against $O_2^{.-}$ generated by MV has been previously described in the works of Banerjee et al. (2013, 2015) (Banerjee et al., 2013, 2015). The advantage of this strain is the versatility of it use, since it will be able to detect a greater variety of contaminants than if it were a specific strain that was

only able to respond specifically to $O_2\cdot^-$ or H_2O_2. However, the specific character of the bioreporters such as *Nostoc* sp. PCC7120 pBG2173, as previously described by Hurtado-Gallego et al., 2018 (Hurtado-Gallego et al., 2018) in the cyanobacterial bioreporters *Nostoc* PCC7120 pBG2154 and *Nostoc* sp. PCC7120 pBG2165, might be also an advantage to detect or classify specific contaminants. A combination of both (specific and unspecific) bioreporters, provides global information about the contamination of environments, allowing an accurate decision on their remediation.

We may also say that both strains are capable to detect the ROS in natural waters. *Nostoc* sp. PCC7120 pBG2173 only detected the $O_2\cdot^-$ formed by MV due to its specific character. However, *Nostoc* sp. PCC7120 pBG2172 was able to detect both ROS. In pristine waters such as Glx1, both compounds were detected within the same order of magnitude than those of nominal concentrations added. In contaminated waters such as WWTP, the detection of bioavailable H_2O_2 by the bioreporters followed the same behaviour, reporting concentrations in the same order of magnitude that those that were nominally added. However, notable differences can be observed in the case of WWTP for MV detection, where the concentrations detected by *Nostoc* sp. PCC7120 pBG2172 were lower than the concentrations nominally added. This type of water has a much more complex and unknown organic composition than pristine waters such as Glx1. The presence of a large amount of organic matter and other contaminants in water could be a factor to be taken into account to explain the lower bioavailability of MV in this type of water due to the formation of complexes between MV and organic matter and other pollutants (Hurtado-Gallego et al., 2018). Given the environmental concentrations of H_2O_2 reported to date (as discussed above), the bioreporter strain *Nostoc* p. PCC7120 pBG2172 was able to detect H_2O_2 in that order of magnitude proving to be a good tool to measure H_2O_2 in real aquatic environments.

In order to validate the bioreporters to detect the ROS formation of emerging pollutants, we used the antimicrobial triclosan. This compound has been previously used in ecotoxicity studies due to its toxicity that supposes a risk in the environment (Rosal et al., 2010a; González-Pleiter et al., 2017). Furthermore, this antimicrobial agent has been described to produce oxidative stress in the organisms (Binelli et al., 2009; Kawanai, 2011; Cherednichenko et al., 2012; Hurtado-Gallego et al., 2018). As can be seen in Fig. 4, both strains responded to triclosan but with different behaviour. For example, *Nostoc* sp. PCC7120 pBG2172 has a higher range of detection, while *Nostoc* sp. PCC7120 pBG2173 is more sensitive to triclosan than *Nostoc* sp. PCC7120 pBG2172 and detect the compound at longer times. These differences indicate the different role of the genes in the detoxification of ROS produced by triclosan (both H_2O_2 and $O_2\cdot^-$).

5. Conclusions

Two cyanobacterial bioreporter strains that respond to oxidative stress have been constructed, which have been denoted *Nostoc* sp. PCC7120 pBG2172 and *Nostoc* sp. PCC7120 pBG2173 by using the promoters of the genes encoding oxidative stress enzymes peroxiredoxin (*2-cys-prx*) and Mn-catalase (*katA*) respectively. The strain *Nostoc* sp. PCC7120 pBG2172 responds to oxidative stress caused by both H_2O_2 and MV ($O_2\cdot^-$) while the strain *Nostoc* sp. PCC7120 pBG2173 only respond to MV ($O_2\cdot^-$). Both cyanobacterial bioreporters are valid for the detection of oxidative stress caused by H_2O_2 and MV (for *Nostoc* sp. PCC7120 pBG2173 only MV) in both pristine and contaminated natural waters. In the case of contaminated water, the bioavailability of MV decreases probably due to the complexation of the compound with organic matter present in the water. Furthermore, both stains detected emerging contaminants, which cause oxidative stress such as triclosan. The great difference between both bioreporters is their specificity, which provide complementary information when a contaminated sample is analysed.

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SUPPLEMENTARY MATERIAL

Peroxiredoxin (*2-cys-prx*) and catalase (*katA*) cyanobacterial-based bioluminescent bioreporters to detect oxidative stress in the aquatic environment

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Table S4. Psychochemical characterization of the natural waters.

Figure S1. Agarose gel electrophoresis of the PCR amplification of *Nostoc* PCC 7120 colonies obtained from the conjugation with pBG2172 (A), from which 14 positive colonies were obtained and C1 and C2 were selected, and pBG2173 (B), from which 7 positive colonies were obtained and C10 and C11 were selected. In both cases (A and B) the first well corresponds to the negative control of the PCR reaction, and the second to the amplification of the corresponding region of the plasmid pBG2146 without insert.

Figure S2. Bioluminescent response of *Nostoc* sp. PCC7120 pBG2146 after the exposure to: A) H_2O_2. Response of *Nostoc* PCC 7120 pBG2146 after 12 hours of exposure to 50 µM and 100 µM of H_2O_2, B) MV. Response of *Nostoc* PCC7120 pBG2146 after 12 hours of exposure to 1200 pM and 1800 pM of MV, C) triclosan. Response of *Nostoc* PCC7120 pBG2146 after 12 hours of exposure to 0.017 mM and 0.025 mM of Triclosan. Parental plasmid pBG2146 bears the T4 transcriptional terminator in order to ensure a minimum baseline luminescence.

Table S1. Main enzymes involved in the protection of *Nostoc* sp PCC 7120 against oxidative stress. Adapted from (Latifi et al. (2009) and Banerjee et al., 2012).

Enzyme type	Enzyme in *Nostoc* sp PCC 7120	Substrate
Superoxide dismutase (SOD)	MnSOD (SodA)	$O_2^{.-}$
Superoxide dismutase (SOD)	FeSOD (SodB)	$O_2^{.-}$
Catalase	Mn-catalase A (KatA)	H_2O_2
Catalase	Mn-catalase B (KatB)	H_2O_2
Peroxiredoxin	Prx-2-cys	H_2O_2, ROOH
Peroxiredoxin	Prx-1-cys	H_2O_2, ROOH
Peroxiredoxin	PrxQ	H_2O_2, ROOH
Peroxiredoxin	Prx II	H_2O_2 + glutathione

Table S2. AA/8 N medium.

Component	Concentration (mM)
KH_2SO_4	2
$MgSO_4$	1
$CaCl_2$	0.5
NaCl	4
Na_2-EDTA	0.077
$FeSO_4$	0.67
Microelements	*

* Microelements: B 4.25 µM, Co 0.17 µM, Cu 0.32 µM, Mn 7.44 µM, Mo 1.25 µM, Zn 0.76 µM, V 0.2 µM

Table S3. Primers for the amplification of the promoter regions of *prx-2-cys* and *katA*. In bold, the sequence recognized by the restriction enzyme is shown; in lower case the bases modified for the generation of the restriction target.

Primer	Sequence 5' - 3'	Restriction site	Size of PCR product (pb)
prx-2-cys Forward	CCAATGC**gt**C**GAC**TTTTCCTGAGA	*Sal*I	523 pb
prx-2-cys Reverse	AACGTA**gg**T**ACC**ACGATAGTCGGA	*Kpn*I	
katA Forward	ATAT**GGTC**g**Ac**TATGACATCCGTTC	*Sal*I	823 pb
katA Reverse	AGTAA**gg**T**ACC**ACTAGAAACAATAAAG	*Kpn*I	

Table S4. Psychochemical characterization of the natural waters.

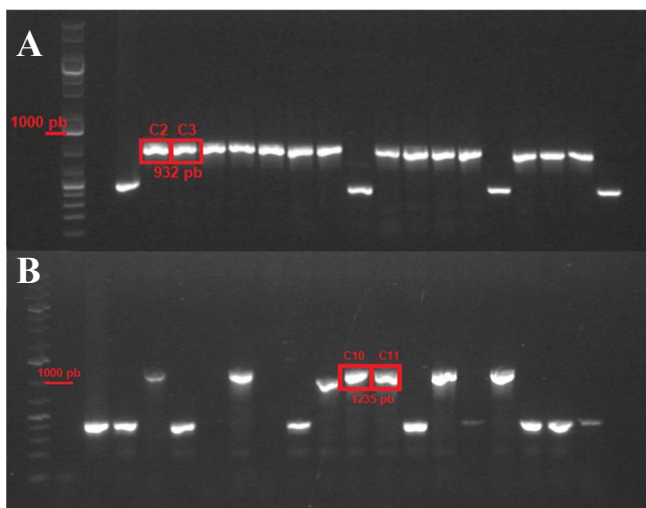| Physicochemical properties	Glx1	WWTP
pH	6.9	7.5
Conductivity (µs/cm)	100	702
Alkalinity (mg/l $CaCO_3$)	14.5	472
PO_{3-4}–P (mg/l PO_4^{3-})	0.05	1.1
Hardness (mg/l $CaCO_3$)	17.7	176
$N-NO_3^-$ (mg/l)	0.2	7
$N-NH_4^+$	0.05	15

Figure S1. Agarose gel electrophoresis of the PCR amplification of *Nostoc* PCC 7120 colonies obtained from the conjugation with pBG2172 (A), from which 14 positive colonies were obtained and C1 and C2 were selected, and pBG2173 (B), from which 7 positive colonies were obtained and C10 and C11 were selected. In both cases (A and B) the first well corresponds to the negative control of the PCR reaction, and the second to the amplification of the corresponding region of the plasmid pBG2146 without insert.
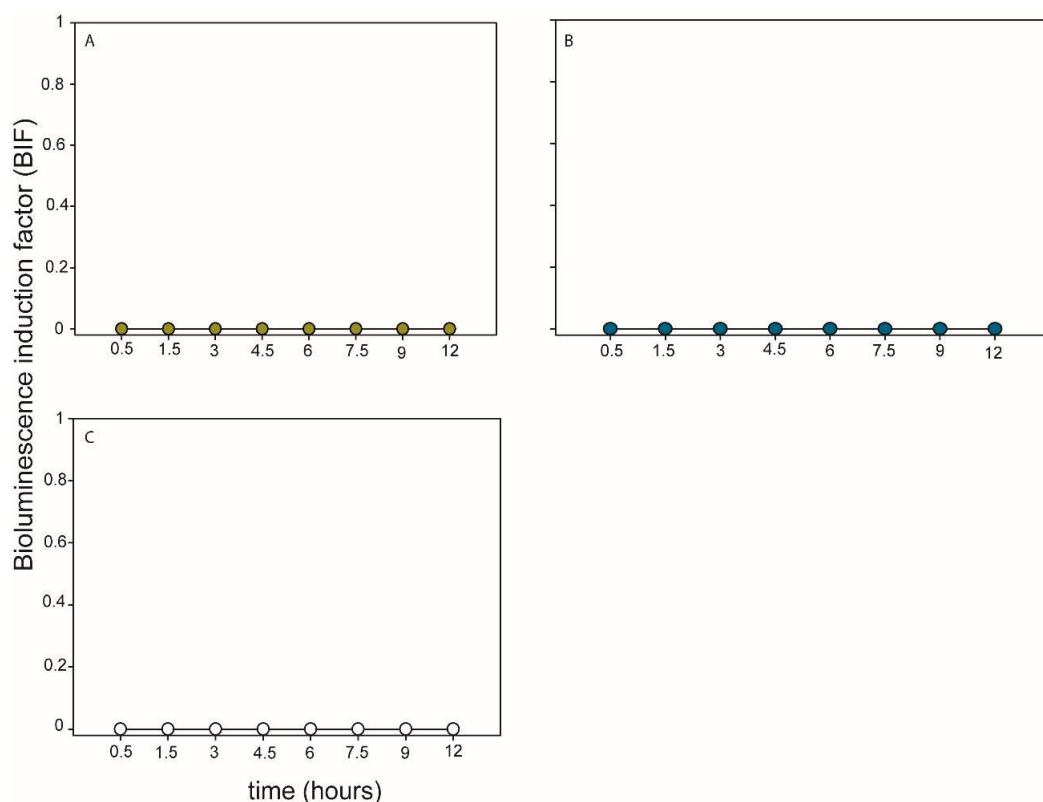

Figure S2. Bioluminescent response of *Nostoc* sp. PCC7120 pBG2146 after the exposure to: A) H_2O_2. Response of *Nostoc* PCC7120 pBG2146 after 12 hours of exposure to 50 µM and 100 µM of H_2O_2, B) MV. Response of *Nostoc* PCC7120 pBG2146 after 12 hours of exposure to 1200 pM and 1800 pM of MV, C) triclosan. Response of *Nostoc* PCC7120 pBG2146 after 12 hours of exposure to 0.017 mM and 0.025 mM of Triclosan. Parental plasmid pBG2146 bears the T4 transcriptional terminator in order to ensure a minimum baseline luminescence.