A Deletion Mutation in the Spacing Within the psaA Core Promoter Enhances Transcription in a Cyanobacterium Synechocystis sp. PCC 6803

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Transcriptional regulation of PSI reaction center psaA is one of the important physiological responses to changing environments. We previously reported that the Rrf2-type transcriptional regulator Slr0846 activates transcription of psaA in Synechocystis sp. PCC 6803. In the Δslr0846 mutant, transcripts from two promoters, P1 and P2, were downshifted and, as a result, a lower Chl content and slower growth were observed. Here, we report spontaneous suppressors which recovered Chl accumulation and photoautotrophic growth. Sequencing of the whole promoter region revealed in some suppressors the same single nucleotide deletion in a 9 bp G stretch (−21 to −29 from the transcriptional start point of P1), which is located between the −35 and −10 elements of the P1 core promoter (hereafter the −G mutation). The transcripts from P1 were higher in abundance in this pseudo revertant than in the Δslr0846 mutant. When the promoter was fused to a reporter gene, the −G mutation conferred ~4 times higher expression than the wild-type promoter. It has been shown that the P1 promoter activity of psaA is regulated by a high light regulatory element 1 just upstream of −35. The −G mutated P1 promoter still retained the high light response. Thus, the −G mutation enhanced the expression level of psaA without a loss of the response to the high light conditions. This is the first study of the spontaneous mutation of a spacer length of a promoter for expression in cyanobacteria.

Keywords: Cyanobacteria • Photosystems • Promoter • Synechocystis sp. PCC 6803 • Transcription.

Abbreviation: HLR1, high light regulatory 1.

Introduction

Photosynthetic organisms must acclimate to various light environments by adjusting photosystems and light-harvesting systems (Kawamura et al. 1979, Melis 1991, Fujita 1997). In cyanobacteria, regulation of the photosystem genes plays a crucial role in the acclimation (Hihara et al. 2001, Singh et al. 2009). Typically, the PSI reaction center gene psaA and many other photosystem genes are highly expressed under low light and are severely downshifted under high light to balance the light and dark reactions, while the PSII reaction center gene psbA is expressed under low light but is upshifted under high light to compensate the photoinhibition. Light responses of psaA have been extensively studied mainly in Synechocystis sp. PCC 6803 (Herranen et al. 2005), and several regulatory mechanisms have been proposed: Slr0846, RpaB and as yet unidentified transcriptional regulators bind upstream of psaA (Muramatsu and Hihara 2006, Seino et al. 2008, Midorikawa et al. 2009).

Previously, we reported that the Rrf2-type transcriptional regulator Slr0846 activates transcription of psaA in Synechocystis (Midorikawa et al. 2009). The psaA and psbB genes are co-transcribed as a single operon from two promoters named P1 and P2, which are located at −144 and −45 from the start codon of psaA, respectively (Eriksson et al. 2000, Muramatsu and Hihara 2006, Midorikawa et al. 2009, Mitschke et al. 2011). Slr0846 binds to a far upstream region of P1 and activates transcription from both P1 and P2. An slr0846-defective mutant showed decreased expression of psaA and a slower growth compared with the wild type.

RpaB is a global transcriptional regulator that responds to high light (Ashby and Mullineaux 1999). A motif called HLR1 (high light regulatory 1), where RpaB specifically binds, is widely found upstream of many photosynthesis genes in cyanobacteria (Eriksson et al. 2000, Kappell and van Waasbergen 2007). RpaB binds to HLR1 under normal light conditions, and is rapidly released in response to high light (Seki et al. 2007, Hanaoaka and Tanaka 2008). In the case of high-light-inducible genes such as psbA, HLR1 partly overlaps their core promoters to allow RpaB to act as a repressor under normal light conditions.

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On the other hand, in the case of light-repressive genes such as \( \text{psaA} \) and many light-harvesting genes, HLR1 is located upstream of the core promoter to allow RpaB to act as an activator under normal light conditions (Seino et al. 2008). There are three HLR1 sequences (named HLR1-A, HLR1-B and HLR1-C) upstream of \( \text{psaA} \) in \textit{Synechocystis}. HLR1-C, which is located adjacent to the core promoter P1, is essential for transcription of \( \text{psaA} \) from P1 (see Fig. 1B) (Muramatsu and Hihara 2006, Takahashi et al. 2010). However, the whole transcript level does not heavily depend on HLR1-C. Moreover, as yet unidentified mechanisms have been postulated for elaborate regulation of \( \text{psaA} \).

As described above, the \( \Delta \text{slr0846} \) mutant grows more slowly than the wild type. After long-term propagation, some pseudorevertants growing better than the original mutant often emerged in the liquid culture and finally took over the entire culture. We isolated several pseudorevertants from such cultures and attempted to identify suppressor mutations, which may recover the downshifted expression of \( \text{psaA} \) genes in the \( \Delta \text{slr0846} \) mutant. In this study, we focused on a suppressor mutant which has a single nucleotide deletion in the spacer between the \( -35 \) and \( -10 \) elements of the \( \text{psaA} \) P1 core promoter. Transcriptional activity of the mutated P1 promoter was higher than that of the wild-type promoter, whereas the high light response of P1 remained unchanged. We conclude from these studies that the mutation in the \( \text{psaA} \) P1 core promoter increases basal transcriptional activity.

### Results

**Isolation and characterization of pseudorevertants from the \( \Delta \text{slr0846} \) mutant**

We isolated several pseudorevertants from the \( \Delta \text{slr0846} \) mutant and confirmed the complete segregation of the \( \text{slr0846} \) disruption (Fig. 1A). Expecting that the suppressor mutation may be located in the cis-element of \( \text{psaA} \), we sequenced the upstream region of \( \text{psaA} \) in five pseudorevertants (named 1R-12, 1R-16, 2R, 3R and 8R). The same single nucleotide deletion was found in a 9 bp G stretch (at position \( -21 \) to \( -29 \) from the transcriptional start point of P1) in three independent clones (1R-12, 1R-16, 2R, 3R and 8R). The deletion is located in a spacer region between putative \( -35 \) and \( -10 \) elements of the P1 core promoter (Fig. 1B). We did not find any other mutation in the upstream region of \( \text{psaA} \), which includes the Slr0846 binding region. Hereafter, we chose one of the pseudorevertants, 1R-16, and used this for further studies (\( -G \) pseudorevertant).

Under normal light conditions, the growth of the \( -G \) pseudorevertant was much faster than that of the parent \( \Delta \text{slr0846} \) mutant but slightly slower than that of the wild type (doubling time of the wild type: \( 12.4 \pm 0.2 \) h, \( \Delta \text{slr0846} \) mutant: \( 30.4 \pm 5.4 \) h, \( -G \) pseudorevertant: \( 16.0 \pm 0.4 \) h) (Fig. 1C). Fig. 2A and B shows the whole-cell absorption spectra and the low temperature Chl fluorescence spectra, respectively. As demonstrated previously (Midorikawa et al. 2009), the \( \Delta \text{slr0846} \) mutant showed a lower Chl content and a lower PSI to PSII ratio than the wild type. The Chl content of the \( -G \) pseudorevertant was mostly recovered (\( \sim 82\% \) of the wild type), while the PSI to PSII ratio remained low (\( \sim 65\% \) of the wild type) (Table 1 and Fig. 2B).
We performed primer extension analysis to study the effect of the \(-G\) mutation on psaA transcription from the two transcriptional start points (Fig. 3). When cells were grown under normal light conditions, transcripts from P1 were much higher in abundance in the \(-G\) pseudorevertant than in the parent \(\Delta sr0846\) mutant, while transcripts from P2 were comparable with those in the parent mutant.

Also, we confirmed the effect of \(-G\) mutation using a reporter gene in the wild-type background. The whole psaA promoters with or without the \(-G\) mutation were fused with \(luxAB\) genes (Fig. 1B) and we measured the activity by RNA gel blot analysis. It was revealed that the transcription activity of the psaA promoter with the \(-G\) mutation was \(-4\) times higher than that of the wild-type promoter (Fig. 4). Thus, we concluded that the \(-G\) mutation in the P1 core promoter indeed upshifted the transcription mainly from P1.

High light response of the \(-G\) mutated promoter

To test whether the \(-G\) mutated promoter still keeps the high light response, we measured the amount of psaA transcripts after short-term high light treatment by primer extension (Fig. 5). In the wild type, the transcripts from both P1 and P2 were quickly down-regulated under the high light conditions, and slowly recovered to certain levels, which were still lower than the level under normal light conditions. Similar down-regulation was also found in the \(-G\) pseudorevertant and the parent \(\Delta sr0846\) mutant, although the recovery of transcription was slow in both strains. These results show that the \(-G\) pseudorevertant retained the high light responses, suggesting that the expression of the \(-G\) mutated promoter was still dependent on RpaB and HLR1.
support the notion that the −G mutation in the P1 promoter and introduction of slr0846 are effective to enhance transcription of psaA. However, the growth of the +slr0846/−G/Δslr0846 strain was not very different from that of the +slr0846/Δslr0846 strain and the wild type under normal light conditions (doubling time of the wild type: 14.8 ± 2.4 h, +slr0846/Δslr0846 strain; 15.5 ± 0.5 h, +slr0846/−G/Δslr0846 strain; 18.9 ± 4.5 h) (Fig. 6D). The PSI to PSII ratio in the +slr0846/Δslr0846 strain was lower than that in the wild type, though the expression of psaA was higher. This might be due to strong expression of slr0846 with the trc promoter. Finally, the PSI to PSII ratio of the +slr0846/Δslr0846 strain was still higher than that of the −G pseudorevertant, despite the fact that their Chl contents were nearly identical (Fig. 6B, C). This may suggest that Slr0846 has extra effects to increase the PSI to PSII ratio in addition to the transcriptional activation of psaA, as suggested previously (Midorikawa et al. 2009).

**Discussion**

In this study, we isolated a pseudorevertant from the slowly growing Δslr0846 mutant and demonstrated that the −G mutation in the psaA core promoter P1 enhanced the transcription and indeed suppressed the Δslr0846 phenotype of reduced Chl accumulation and slow growth. This is the first report of a mutation in the psaA core promoter which specifically recovered the defect in phototrophic growth. It is also surprising that the wild-type psaA promoter, which is one of the strongest promoters in cyanobacteria, is upshifted by the −G mutation. Thus, the enhanced psaA promoter could be useful for overexpression of genes of interest in biotechnology such as photosynthetic biomass production.

Generally, transcription of bacterial genes is often regulated by specific transcription factor(s), a repressor and/or activator. The expression level is also determined by basal promoter activity, which reflects the affinity of the RNA polymerase holoenzyme for the core promoter (Herring et al. 2005). Since the typical σ factor in the holoenzyme recognizes both the −10 and −35 elements in the core promoter, not only the similarity of each element to the consensus but also the spacer length between the −10 and −35 elements are critical for the affinity of the holoenzyme for the promoter (Dombroski et al. 1996). In the case of the principal σ factor σ^70 of E. coli, the optimal spacer length has been estimated as 17 ± 1 bp (Russell and Bennett 1982). Similar optimal spacing of approximately 18 bp has been reported experimentally in cyanobacteria (Elhai 1993, Mazouni et al. 1998). In fact, the principal σ factor SigA of cyanobacteria is homologous to σ^70 of E. coli (Gruber and Gross 2003). As for the psaA P1 promoter, we provisionally determined the −10 and −35 elements as in Fig. 1B, according to the transcriptional start point and consensus of E. coli σ^70, although the −35 sequence of the cyanobacterial promoter is often weakly conserved (Vogel et al. 2003). It is thus presumed that a 1 bp deletion in the spacer region between the

**Physiological effects of the −G mutated psaA promoter**

To study the physiological significance of the −G mutation, we measured psaA expression and the PSI to PSII ratio of the −G mutation on the wild-type background. First, we attempted to introduce the −G mutation with an antibiotic-resistant cassette using the construct shown in **Supplementary Figure S1**. However, we only obtained transformants carrying the wild-type promoter perhaps due to an insufficient homologous region downstream of the −G mutation. More transformants should be examined to screen the −G promoter. Secondly, we introduced a second copy of slr0846 at a neutral site in the −G pseudorevertant derived from the slr0846 mutant (named + slr0846/−G/Δslr0846) and into the Δslr0846 mutant as a control (named + slr0846/Δslr0846). As a result, the expression level of psaA was recovered in the +slr0846/−G/Δslr0846 strain and the +slr0846/Δslr0846 strain, by introduction of slr0846. Obviously, the transcript level in the +slr0846/−G/Δslr0846 strain was higher than that in the +slr0846/Δslr0846 strain, although the transcripts in both strains were more abundant than in the wild type (Fig. 6A). Consistently, the Chl content of cells grown under normal light conditions was also higher in the +slr0846/−G/Δslr0846 strain than in the +slr0846/Δslr0846 strain and the −G pseudorevertant (−G/Δslr0846) (Fig. 6B). Similar results were observed for the PSI to PSII ratio, which was estimated by the low temperature Chl fluorescence spectra of the cells (Fig. 6C). These findings...
−10 and −35 elements enhanced the basal core promoter activity of P1.

Regulation of the spacer has been known in some genes. In E. coli, the stress-inducible soxS gene has a suboptimal 19 bp spacer between the −10 and −35 elements of its promoter, and a 1–3 bp deletion in the spacer resulted in enhanced expression of soxS (Hidalgo and Demple 1997). In this case, the physiological regulation of soxS expression is mediated by specific interaction of superoxide-responsive SoxR protein with the spacer, leading to distortion of the suboptimal spacer to generate a better promoter (Watanabe et al. 2008). Similar distortion of the spacer may also regulate the expression of psaA in Synechocystis under certain conditions to require more PSI.

Another explanation of the effect of the −G mutation may be that the enhanced transcription of psaA was mediated by RpaB, since the RpaB-binding site, HLR1-C, is located at −42 to −60, which is 1 bp closer to the core promoter in the −G pseudorevertant than in the wild type. It is generally thought that the bound RpaB protein interacts with the RNA polymerase at −35/−10 elements. The closer distance may well enhance the interaction and may lead to upshift of the psaA transcription. However, the high light response of psaA was not affected by the −G mutation. As a third possibility, transcription of psaA might be regulated by an as yet unknown repressor that binds to the guanine stretch like SoxR, although such a protein has not been detected (Muramatsu and Hihara 2006).

The −G mutation on the nearly wild type background (+slr0846/Δslr0846) led to a higher Chl content per cell and a higher PSI to PSII ratio than the wild type under normal light conditions. On the other hand, introduction of an additional psaA−psaB copy with a high-light-inducible promoter of psbAII into wild-type Synechocystis did not elevate the PSI content in the cells grown under normal light conditions but retarded the growth under high light conditions (Muramatsu et al. 2009). Since expression of psaA−psaB must be severely down-regulated under high light (Hihara et al. 1998, Hihara et al. 2001), inappropriate regulation of psaA−psaB expression may be responsible for the growth retardation under high light. In contrast, the core promoter with the −G mutation allows enhanced expression of PSI under normal light, but still keeps the high-light-inducible suppression. Thus, the mutant core promoter in this communication provides a unique tool for expression under low or normal light conditions.

We found the single base deletion in the nine contiguous guanine bases within the spacer region in several independent suppressor clones. However, no deletion has yet been found in the other part of the spacer. These results suggest that the contiguous guanine bases may be a hotspot for mutation. A similar mutation is found in the eight contiguous guanine bases of pilC in Synechocystis and Thermosynechococcus elongatus BP-1 (Bhya et al. 2000, Nakamura et al. 2002), i.e. one guanine base is inserted in the same 8 bp guanine stretch in the coding region of pilC in both sequenced strains of Synechocystis and T. elongatus. The same inactivation of pilC in the two species strongly supports the hotspot for mutation in the 8 bp guanine stretch. Since PilC is essential for biogenesis of type IV pili, strains lacking pili-dependent cell motility may have been fortuitously selected in laboratories. In Prochlorococcus marinus MED4, it is reported that a 1 bp deletion mutation was found in a 6 bp adenine stretch in the upstream region of the mutT-phrB operon in several independent UV-hyper-resistant variants, where this deletion seems to upshift transcription of mutT and phrB, which encode DNA repair enzymes (Osbourne et al. 2010). Although the transcriptional start site of this operon was not determined, the 6 bp stretch might be located in a spacer of the −35 and −10 elements. These facts suggest that a homopolymeric stretch of DNA may be error prone because of slippage in DNA replication (Streisinger and Owen 1985, Hoffmann et al. 2003). For further confirmation of such a mutation, we may screen a +G insertion.

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Fig. 5 Analysis of the high light response of psaA by primer extension. Cell cultures were transferred from normal light (25 μmol m⁻² s⁻¹) to high light (200 μmol m⁻² s⁻¹) for 30, 180 and 360 min. WT, wild type; Δ, the Δslr0846 mutant; −G/Δ, the −G pseudorevertant. Arrows indicate the transcriptional start points (P1 and P2) and numbers indicate positions relative to P1.
mutation to reduce the elevated expression of \textit{psaA} in the \textit{slr0846} mutant.

There have been many reports that deal with suppressor mutation in various mutants of cyanobacteria. One good example of such studies is a suppressor of an iron–sulfur cluster point mutant, which has a directed cysteine to serine mutation in \textit{PsaC} of PSI (Yu et al. 2003). The suppressor mutation was identified in a novel repressor gene \textit{sufR} that normally represses expression of \textit{suf} genes, which encode general assembly factors for the iron–sulfur clusters (Wang et al. 2004). Another example is a suppressor of a \textit{psbV} disruptant which does not grow photoautotrophically in the absence of Ca\textsuperscript{2+} or Cl\textsuperscript{−} ions (Kobayashi et al. 2006). Since \textit{psbV} encodes a PSI extrinsic protein, Cyt c550, the requirement for Ca\textsuperscript{2+} and Cl\textsuperscript{−} ions has been interpreted as Cyt c550 concentrating these ions at the donor side of PSI (Shen et al. 1998). The suppressor of the \textit{psbV} mutant was found to have insertional inactivation of a novel anion transporter \textit{Slr0753}, suggesting that \textit{Slr0753} exports Cl\textsuperscript{−} ions from the thylakoid lumen. Thus, studies of suppressor mutations often provide us with a new research approach, although the molecular mechanisms of the suppressors are not always clear. On the other hand, suppressor mutations from a transcriptional activator mutant are likely to compensate the decreased expression of its target genes. In the case of the \textit{slr0846} mutant, which showed decreased expression of \textit{psaA}, we may expect a suppressor mutation in the \textit{psaA} promoter or in a corresponding regulator(s) to recover the expression. Indeed, we found the -\textit{G} mutation in the core promoter to upshift the expression level of \textit{psaA}. We assume that such an upshifting suppressor mutation is rather rare in contrast to...
common inactivation mutations. We also found that as yet unknown suppressor mutations recovered the psaA expression. Since a negative regulator is hypothesized to bind to the HNE2 element in the psaA promoter (—272 to —178), those unknown mutations may occur in such a negative regulator (Muramatsu and Hihara 2006). Further survey of the other suppressors would provide us with clues for the complex regulation of psaA.

Materials and Methods

Strains and growth conditions

The original motile strain of Synechocystis sp. PCC 6803 showing positive phototaxis was used as the wild type (Ikeuchi and Tabata 2001). As previously described, the slr0846 gene was disrupted by insertion of the chloramphenicol resistance gene derived from pACYC184 (Midorikawa et al. 2009). The disruption of slr0846 in the pseudorevertant was confirmed by PCR with primers, slr0846-1 5’-GGATGTTCCCTTTAATT-3’ and slr0846-3 5’-GGTGCCAAAAGACCCACGC-3’ (Midorikawa et al. 2009). The wild type and mutant were grown at 30°C in BG11 medium supplemented with 20 mM HEPES-KOH (pH 7.8) (Rippka 1988) with bubbling of 1% (v/v) CO2 under continuous illumination with white fluorescent lamps (20–30 μmol m−2 s−1, FL20SSW/18; Mitsubishi). Chloramphenicol, spectinomycin dihydrochloride pentahydrate or kanamycin sulfate was added at a concentration of 20 μg ml−1 to maintain the strains, when required. All strains were grown without drugs when measurements were done. Cell density was monitored as optical density at 730 nm with a spectrophotometer (Model UV-2400PC; Shimadzu). Cells at log phase were harvested and resuspended at a cell density of OD730 = 2.0. The 77 K fluorescence spectra were recorded using a spectrofluorometer (RF-5300PC; Shimadzu). Doubling time, expressed as an average with the standard deviation, was calculated from mid log phase data (OD730 = 0.1–1.0) in three independent experiments.

Analyses of the photosynthetic parameters

The Chl content was calculated after extraction with 100% methanol as described (Grimme and Boardman 1972). Absorption spectra were recorded using a spectrophotometer (model U-3500S; Hitachi) equipped with an end-on photomultiplier. Cells at log phase were harvested and resuspended at a cell density of OD730 = 2.0. The 77 K fluorescence spectra were recorded using a spectrofluorometer (RF-5300PC; Shimadzu). Cells at log phase were harvested and resuspended at 5 μg Chl ml−1. After dark adaptation for 10 min, cells were frozen in liquid N2. The bandwidth of the excitation light was 10 nm.

RNA isolation

Cells at mid-log phase (OD730 ~0.4) were collected by centrifugation at 6000 × g for 10 min at 4°C and stored in liquid N2. The frozen cells were thawed with 500 μl of a buffer containing 10 mM Tris–HCl (pH 8.0), 1 mM EDTA and 1% (w/v) SDS at 65°C and immediately treated with 500 μl of phenol at 65°C for 15 min. After centrifugation, the supernatant was extracted with phenol/chloroform, then chloroform, and finally precipitated in ethanol. To eliminate trace amounts of contaminating DNA, RNA samples were incubated with RNase-free DNase I (TAKARA) for 30 min at 37°C. After extraction with phenol/chloroform, then chloroform, and precipitation with ethanol, RNA was dissolved in water.

Primer extension analysis

Total RNA (10 μg) was subjected to primer extension assays using a fluorescein isothiocyanate (FITC)-labeled primer specific to psaA (5’-GGCCCTAGGCTCTTCGGC-3’) (ESPEC Oligo Service). Total RNA was incubated at 95°C for 5 min with 1 pmol of the labeled primer. Then, SuperScript II Reverse Transcriptase (1.5 U; Invitrogen) and first-strand buffer (20 mM Tris–HCl pH 8.4, 10 mM MgCl2, 1.6 mM dNTPs) were added and the reaction mixture was incubated at 42°C for 1 h. The products were ethanol precipitated, resuspended in loading buffer [89% (v/v) formamide, 1.5% (w/v) blue dextran and 1 mM EDTA] and denatured at 95°C for 5 min. Samples were analyzed on a 4% (w/v) polyacrylamide–urea gel using a DNA sequencer DSQ-2000L (Shimadzu). The sequencing ladder was obtained using the EXCEL II DNA sequencing kit-LC (Epicentre Technologies) with the same primer as that used for the primer extension analysis.

RNA gel blot analysis

RNA gel blot analyses were performed as described (Islam et al. 2008). A 1 μg of total RNA was loaded for each lane. The probes for psaA and luxAB were obtained by amplification of the coding region by PCR using primers 5’-GGATCGGATGCCTGC GCCAC-3’ and 5’-CTAGCAATGGGAAGACTGC-3’ for psaA and 5’-TGACACGTTGGCTTACC3’ and 5’-TTACAGTGTGTATTTGACG-3’ for luxAB.

Construction of plasmids

The slr0846 gene was inserted with the trc promoter and the N-terminal His-tag into a neutral site of Synechocystis sp. PCC 6803. In detail, a 1.9 kb platform DNA containing sll0822, slr0846 and a part of psaA for double recombinant (Satoh et al. 2001), and a kanamycin resistance cassette, was excised with NdeI and BamHI and subcloned into the expression vector pTKH2031V, which has a trc promoter, a platform sequence of slr0846 for double recombinant (Satoh et al. 2001), and a kanamycin resistance cassette instead of a chloramphenicol resistance cassette in pTCH2031V.

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(Ishizuka et al. 2006). The resulting plasmid was digested with Dral, and a 2.5 kb fragment containing the kanamycin resistance cassette, trc promoter and slr0846 was extracted and cloned into the BsaBI site of the platform DNA in pPCR-Script-slr0846-1/9 (named pTKHT0846-slr0846).

All plasmids for promoter analysis were derivatives of pCFS, which allows introduction of a promoter and luxAB genes just after the ndhB gene by double homologous recombination (Aoki et al. 1995). Each promoter fragment was generated by PCR with primers (psaA-6 S'-ccagctgAAGACTGCGCTG TCC-3' and psaA-14 5'-gggatccGTATGGGTGTCCTCC CGC-3') and cloned into the pT7blue T-vector. After verification of the direction, all inserts were excised from the plasmid with BamHI and cloned into the unique BamHI site of pCFS to produce transcriptional fusions with the luxAB genes. The nucleotide sequence and direction of the promoter in these reporter constructs were verified by sequencing.

**Supplementary data**

Supplementary data are available at PCP online.

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