Antitoxin MqsA Represses Curli Formation Through the Master Biofilm Regulator CsgD

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MqsA, the antitoxin of the MqsR/MqsA toxin/antitoxin (TA) system, is a global regulator that reduces expression of several stress response genes (e.g., mqsRA, cspD, and rpoS) by binding to the promoter palindromic motif [5’-AACCT (N)₃ AGGTT-3’]. We identified a similar mqsRA-like palindrome [5’-AACCT TA AGGTT-3’] 78 bp upstream of the transcription initiation site in the csgD promoter (p-csgD). CsgD is a master regulator for biofilm formation via its control of curli and cellulose production. We show here that MqsA binds to this palindrome in p-csgD to repress csgD transcription. As expected, p-csgD repression by MqsA resulted in reduced transcription from CsgD-regulated curli genes csgA and csgB (encoding the major and minor curli subunits, respectively). Curli production was reduced in colonies and in planktonic cells upon MqsA production. Hence, MqsA directly represses p-csgD, and thereby influences curli formation. This demonstrates that TA systems can impact overall cell physiology by fine-tuning cellular stress responses.

Biofilms consist of bacterial populations adherent to each other, and often, to solid/liquid or air/liquid interfaces. In Escherichia coli and Salmonella spp., a major extracellular component that promotes biofilm formation is curli. Curli are thin proteinaceous, amorphous fibers (usually 4–12 nm in width and 100 to 10,000 nm in length) that were initially identified as a surface organelle in E. coli that binds to host fibronectin. Secretion of curli fibers to the bacterial membrane surface requires seven genes in two adjacent divergently transcribed operons: csgDEFG and csgBAC. CsgE, CsgF, and CsgG are accessory membrane proteins for efficient curli secretion, while CsgB and CsgA are structural subunits (curlin) that assemble into mature curli fibers. The role of CsgC in curli biogenesis is less understood, although it has been suggested to participate in redox activity with CsgG. In E. coli, both operons are activated by CsgD, a transcriptional regulator belonging to the FixJ/UhpA/LuxR family. In addition to curli expression, CsgD also transcriptionally activates the gene of diguanylate cyclase AYcE, which synthesizes cyclic diguanylate (c-di-GMP) acids. Both AdrA and c-di-GMP have been implicated in cellulose production. As both curli and cellulose are components in biofilms, csgD regulation is thus an important determinant in microbial adaptation to different environments.

Curli production is highly responsive to environmental fluctuations such as low temperature, low osmolarity, and nutrient limitation. These environmental cues influence the expression of no less than 10 transcriptional factors, which in turn regulate the expression of csgD. For instance, csgD is activated by RNA polymerase containing the stationary phase sigma subunit σ₇ (RpoS) during stationary growth phase. This activation is further amplified by a positive feedback loop through CsgD-dependent transcription of iraP, which encodes a stabilizing factor for RpoS. Another transcriptional factor, MlrA, also stimulates CsgD expression through a signaling cascade of c-di-GMP generated by YegE and YdaM. At the post-transcriptional level, the 5’ untranslated region of csgD mRNA is also a regulatory hotspot. At least four small RNAs [McaS, RprA, and OmrA, OmrB] can directly bind to the 5’ untranslated region of csgD to subsequently inhibit its expression. Overall, the multiple regulatory layers for CsgD expression underlie the complex regulation of curli production and biofilm formation.

Bacterial toxin/antitoxin (TA) systems are genetic elements that encode both toxic proteins that disrupt cellular processes, and antitoxins that attenuate this toxicity. TA systems are prevalent, with at least 38 TA systems identified in E. coli alone. Among these, the MqsR/MqsA system is notable for its involvement in persistence, quorum sensing, biofilm formation, direct control of another TA system, and global regulation through the MqsR toxin and the MqsA antitoxin. MqsA possesses a C-terminal helix-turn-helix domain that allows...
Figure 1 | The csgD promoter (p-csgD) region. The black boxes indicate the −35 and −10 promoter regions. The mqsRA-like palindrome that contains the 5′-AACCT (N)_3 AGGTT-3′ motif is highlighted in green (at position −78 relative to the transcriptional start site) with the spacer in yellow. The sequence in blue was used as the DNA probe (p-csgD) for EMSA. Nucleotides in bold indicate the binding site for H-NS, while those boxed in red indicate the binding sites for IFH. Nucleotides with a wavy underline indicate the binding site for CpxR.

Results

We have shown that MqsA is a global regulator that represses rpoS transcription by binding to the mqsRA-like palindrome of p-rpoS[17]. RpoS is the master regulator of stress response[35]. Upon binding to this palindrome through its C-terminal domain, MqsA controls expression of various genes such as mqsRA[17], rpoS[35], and cspD[34].

Previously, we identified an mqsRA-like palindromic motif [5′-AACCT TA AGGTT-3′] in the promoter of csgD using a whole-genome bioinformatic search[31]. Here, we show that MqsA binds to this mqsRA-like palindrome to repress csgD expression, which eventually results in reduced curli formation in E. coli. Taken together, MqsA behaves as a negative determinant in biofilm formation and as a regulator of an important regulator (CsgD).

Curli production is reduced in MqsA-producing strains. As Congo Red (CR) is a dye that binds curli and cellulose[38], colonies with high amounts of curli will appear red on salt-free agar. Note that E. coli K12 does not produce cellulose[13,37]. As expected, the csgD deletion strain appeared as white colonies (Fig. 2a). In an mqsRA deletion strain, producing MqsA from a plasmid rendered the colonies less red than an isogenic strain harboring the empty plasmid after prolonged incubation (Fig. 2a). By quantifying the amount of CR bound to planktonic cells, we determined that curli production was 1.9 ± 0.2 fold and 1.7 ± 0.2 fold less in the MqsA-producing cells after 3 h and 6 h incubation, respectively (Fig. 2b). Therefore, MqsA reduces CsgD activity via two pathways: (i) indirect repression of p-csgD through repression of p-rpoS under oxidative stress; and (ii) direct repression of p-csgD in the absence of oxidative stress.

To corroborate these results, we further examined the content of curli at cellular level using SEM. In cells harboring the empty plasmid, curli fibers were present (Fig. 3, left panel) in considerable amounts after 2 days of incubation, with curli forming extracellular matrix that traps individual cells to form biofilms. Cells also showed a rougher surface with tiny lumps. In contrast, curli were essentially absent in MqsA-producing cells harboring empty plasmid. Hence, production of MqsA reduces curli production. Given that curli production is positively correlated with biofilm formation, this reduced amount of curli in MqsA-producing cells is consistent with the previous observation that biofilm formation was decreased by 2 fold in cells expressing MqsA[31].

Curli-related gene transcripts are reduced in cells expressing MqsA. Since p-csgD is repressed by MqsA, we reasoned that genes controlled by CsgD, such as csgB (curli-related) and adrA (cellulose-related), will also be repressed upon production of MqsA. To investigate this possibility, we tested the expression of csgB and adrA in various growth conditions using quantitative real-time reverse-transcription PCR (qRT-PCR). csgB encodes the minor curli subunit, while adrA (encoding a cyclic diguanylate) is part of the regulatory network in cellulose production. In the five growth conditions tested, the csgD transcript in MqsA-expressing cells, whose mqsRA loci were deleted, was consistently decreased by 2 to
manner\(^{31}\), which further implies that curli and cellulose production are regulated differently during biofilm formation.

To demonstrate the direct effect of MqsA on p-csgD, we tested the expression of csgD, csgB, and adrA in an rpoS-deleted strain. In the absence of RpoS, transcription of csgD, csgB, and adrA in MqsA-overproducing cells remain repressed (Table 3). In comparison with an rpoS\(^+\) strain with MqsA produced from plasmid (BW25113 ΔmqsRA/pBS(Kan)-mqsA), the csgD, csgB, and adrA transcripts were repressed \(-50\%\) less in the rpoS-deleted strain with MqsA produced.

To corroborate this direct binding of MqsA to p-csgD, we produced MqsA via pCA24N-mqsA in MG1655 Δ6 R3 PrpoS that harbors a mutated mqsRA-like palindrome (5'-ACCT TGC TAC-3') upstream of chromosomal rpoS\(^{53}\), and measured the transcription of csgD, csgB, and adrA. In this background, MqsA is unable to affect chromosomal rpoS transcription due to the mutated palindrome in the rpoS promoter\(^{53}\). In comparison to the isogenic strain harboring an empty plasmid, csgD, csgB, and adrA were reduced by \(-2\) fold upon MqsA production (Table 3). csgD transcription was further repressed by nearly 5 fold in MG1655 Δ6 R1 PrpoS, a strain that harbors the wild-type mqsRA-like palindrome (5'-ACCT TGC AGGT-3') in the rpoS promoter\(^{53}\). Hence, MqsA represses csgD transcription in the absence of its effect on rpoS transcription, and there is a greater reduction in transcription of csgD when both the promoter of rpoS and csgD are repressed. Therefore, these results confirm direct p-csgD repression by MqsA and demonstrate that

Figure 2 | MqsA decreases EPS production. (a) Colony morphology of strains grown on salt-free CR plates containing 1 mM IPTG for 7 days. Red color indicated curli/cellulose production and scale bars represent 1 cm. Empty vector: BW25113 ΔmqsRA/pBS(Kan); MqsA: BW25113 ΔmqsRA/pBS(Kan)-mqsA; and ΔcsgD: BW25113 ΔcsgD. (b) The amount of Congo red bound to planktonic cells at various time points. Error bars denote standard deviation (n = 2).

6 fold, in comparison to cells with the empty plasmid (Table 3). Similarly, csgB and adrA transcripts were also reduced under the same conditions. The largest reductions in csgD and csgB transcripts were seen with prolonged MqsA overexpression; in particular, after \(-6\) h of MqsA overexpression in LB, csgD and csgB were repressed by nearly 6 fold and 109 fold, respectively. In contrast, adrA repression was more apparent when MqsA production was induced for a short duration. Under 30 min induction, adrA transcript was decreased by nearly 5 fold, but the reductions were less than 3 fold under long inductions (>1 h). This suggests that CsgD does not activate csgB and adrA in the same manner\(^{31}\), which further implies that curli and cellulose production are regulated differently during biofilm formation.

To demonstrate the direct effect of MqsA on p-csgD, we tested the expression of csgD, csgB, and adrA in an rpoS-deleted strain. In the absence of RpoS, transcription of csgD, csgB, and adrA in MqsA-overproducing cells remain repressed (Table 3). In comparison with an rpoS\(^+\) strain with MqsA produced from plasmid (BW25113 ΔmqsRA/pBS(Kan)-mqsA), the csgD, csgB, and adrA transcripts were repressed \(-50\%\) less in the rpoS-deleted strain with MqsA produced.

To corroborate this direct binding of MqsA to p-csgD, we produced MqsA via pCA24N-mqsA in MG1655 Δ6 R3 PrpoS that harbors a mutated mqsRA-like palindrome (5'-ACCT TGC TAC-3') upstream of chromosomal rpoS\(^{53}\), and measured the transcription of csgD, csgB, and adrA. In this background, MqsA is unable to affect chromosomal rpoS transcription due to the mutated palindrome in the rpoS promoter\(^{53}\). In comparison to the isogenic strain harboring an empty plasmid, csgD, csgB, and adrA were reduced by \(-2\) fold upon MqsA production (Table 3). csgD transcription was further repressed by nearly 5 fold in MG1655 Δ6 R1 PrpoS, a strain that harbors the wild-type mqsRA-like palindrome (5'-ACCT TGC AGGT-3') in the rpoS promoter\(^{53}\). Hence, MqsA represses csgD transcription in the absence of its effect on rpoS transcription, and there is a greater reduction in transcription of csgD when both the promoter of rpoS and csgD are repressed. Therefore, these results confirm direct p-csgD repression by MqsA and demonstrate that

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**Table 1 | Bacterial strains and plasmids used in this study**

| Strains or plasmids | Description | Source      |
|---------------------|-------------|-------------|
| **E. coli K-12**    |             |             |
| BW25113             | lac\(^+\) mB14 ΔlacZ\(_{W_{16}}\) hsdR514 ΔaraBAD\(_{H_{33}}\) ΔrhaBAD\(_{D_{78}}\) | 53, 34, 53 |
| BW25113 ΔmqsRA      | BW25113 ΔmqsRA ΔKm\(^+\) | 34, 53 |
| BW25113 ΔcsgD       | BW25113 ΔcsgDΔΩ Km\(^+\) | 31 |
| BW25113 ΔrpoS ΔKm\(^+\) | BW25113 ΔrpoS ΔKm\(^+\) | 31 |
| MG1655 Δ6 R1 PrpoS  | MG1655 ΔmazEF ΔrelBEF ΔchpB ΔyeFM-yoeB ΔdinJ-yafQ ΔmqsRA ΔlacZYA ΔΩ Km\(^+\) | 53 |
| MG1655 Δ6 R3 PrpoS  | MG1655 ΔmazEF ΔrelBEF ΔchpB ΔyeFM-yoeB ΔdinJ-yafQ ΔmqsRA ΔlacZYA ΔΩ Km\(^+\) | 53 |
| Plasmids            |             |             |
| pBS(Kan)            | Km\(^+\) | 34, 52, 54 |
| pBS(Kan)-mqsA       | Km\(^+\), P\(_{lac}::mqsA\) | 34 |
| pCA24N              | Cm\(^+\), lac\(^+\) | 34, 52 |
| pCA24N-mqsA         | Cm\(^+\), lac\(^+\), P\(_{lac}::mqsA\) | 34, 52 |
| pCP20               | Ap\(^+\), Cm\(^+\), FLP\(^+\), λ C857\(^+\), λ pR Rep\(^+\) | 34, 54 |

Km\(^+\), Ap\(^+\), Cm\(^+\), and FLP\(^+\) denotes kanamycin, ampicillin, chloramphenicol and tetracycline resistance, respectively.
MqsA binds the mqsRA-like palindrome in p-csgD. To investigate whether MqsA binds the mqsRA-like palindrome in p-csgD to mediate gene repression, a 312-bp fragment (p-mqsRA) was amplified from the csgD promoter of E. coli (Fig. 1), and incubated with MqsA in EMSA reactions. We found that MqsA binds p-csgD (Fig. 4a, lane 2), and that this binding could be reversed by adding unlabeled p-csgD in excess (Fig. 4a, lane 3). For the positive control, MqsA bound the p-mqsRA double palindrome and formed three distinct bands: the most prominent and largest DNA-MqsA band is where MqsA mediates CsgD repression.

We investigated the specificity of the MqsA binding to p-csgD at the mqsRA-like palindrome by incubating MqsA with a 30-bp fragment that corresponds to either the native mqsRA-like palindrome (5'-AACCTTAAGGTT-3') or its mutated counterpart with five nucleotides changed and is not able to form a palindrome (5'-AACCTTAACGTT-3') (Table 2). MqsA-bound native palindromes in p-csgD were shifted upon adding a 50-fold, a 100-fold or a 200-fold excess MqsA (Figure 4b, lanes 4, 6, 8). However, when the p-csgD mutated palindrome was used, the binding was drastically reduced (Figure 4c, lanes 4, 6, 8). This shows that MqsA binding to the mqsRA-like palindrome in p-csgD is specific, and this binding mediates CsgD repression.

**Discussion**

Elucidating the synthesis of bacterial curli amyloids, and its regulation, is important for biofilm research, particularly from a clinical perspective. Bacterial curli fibers share structural, biochemical and biophysical properties with protein amyloids\(^\text{39}\), which are

**Table 2 | Oligonucleotides used for qRT-PCR, EMSA and for verification of chromosomal mutants**

| Primer name | Sequence (5' → 3') |
|-------------|-----------------|
| **qRT-PCR** |                   |
| rnsGf       | TATTCGAAATGGGCGCAAG |
| rnsGr       | ACTAAACAAAACGCCTCGGT |
| csgDf       | ATACCGCTGAAAGATTACCGTG |
| csgDr       | AGTAAAGGGGCTGATTCCGTG |
| csgBf       | TCACGGACCTAATTCTGCAAG |
| csgBr       | CATAAGCACCTTGCGAAATCG |
| adrA†       | ACACGATACGCGGCGTGATATA |
| **EMSA**    |                   |
| EMSA-csgDf  | CAAATGTCGACCTTCTCA |
| EMSA-csgDr  | AGTATGACCTGAAATACTA |
| p-mqsRf     | GTGGATGCTGACTCCAGCTT |
| p-mqsRr     | CGTGTATGTGGCTGCGTT |
| mqsAepf     | GAGCTAGTACTGTCGACTAAGGAAAGATAATG |
| mqsA-Nr     | GGTAAGAGAGAAAGTTTTTCGAACCTTCAC |
| pCsgDf      | ACTATAAAGACCTTAAGGTTAACATTTA-bio |
| pCsgDr      | TAAATGTTCAGCTAAAGGTTAACATTTA-bio |
| pCsgDm     | ACTATAAAGACCTTAAGGTTAACATTTA-bio |
| pCsgDr-m    | TAAATGTTCAGCTAAAGGTTAACATTTA-bio |
| **Verification of chromosomal mutants** |
| CmqsRAf     | GTGGATGCTGACTTCCGTAAC |
| CmqsRAn     | TCCAGTATCTCCAGCGGTCAG |
| rpsoF       | AAATCAGGCGGAACCCGCTTCG |
| ygBNr       | CTGTTCCGCCGTAATTGAGC |

All oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, IA). "f" indicates forward primer and "r" indicates reverse primer. For EMSA oligonucleotides, the mqsRA-like palindrome is underlined, and the mutated nucleotides are boxed. "bio" indicates that the oligonucleotide is biotin-labeled at the 3' end.

**Table 3 | Summary of qRT-PCR results**

| Condition | Host | OD<sub>600</sub> at induction | Growth medium | Induction duration | csgD | csgB | adrA |
|-----------|------|-------------------------------|---------------|-------------------|------|------|------|
| **Plasmids:** pBS[Kan]-mqsA vs. pBS[Kan] |      |                              |               |                   |      |      |      |
| BW25113 ΔmqsRA | 1.0 | LB                            | 1.0 h         | −3.4 ± 1.2        | −3.0 ± 1.3 | −2.6 ± 1.2 |
| BW25113 Δpso ΔKm<sup>+</sup> | 1.0 | LB                            | 1.0 h         | −1.5 ± 1.2        | −1.4 ± 1.3 | −1.4 ± 1.2 |
| BW25113 ΔmqsRA | 1.0 | LB                            | 5.5 h         | −5.7 ± 1.4        | −10.9 ± 1.4 | −1.1 ± 1.5 |
| BW25113 ΔmqsRA | 1.0 | Salts-free LB                 | 5.5 h         | −1.6 ± 1.4        | −3.6 ± 1.4 | −1.2 ± 1.4 |
| BW25113 ΔmqsRA | 6.0 | Salts-free LB                 | 0.5 h         | −2.9 ± 1.4        | −3.2 ± 1.4 | −4.6 ± 1.4 |
| BW25113 ΔmqsRA | 0.5 | M9/glucose + 2.5% LB<sup>12</sup> | 1.0 h       | −3.2 ± 1.1        | −3.0 ± 1.2 | −2.4 ± 1.2 |
| **Plasmids:** pCA24N-mqsA vs. pCA24N |      |                              |               |                   |      |      |      |
| MG1655 Δ6 R1 pPso | 0.5 | LB                            | 3.0 h         | −4.7 ± 1.1        | −1.7 ± 1.2 | −1.7 ± 1.3 |
| MG1655 Δ6 R3 pPso | 0.5 | LB                            | 3.0 h         | −2.1 ± 1.3        | −2.0 ± 1.2 | −1.9 ± 1.2 |

Means and standard deviations for duplicate reactions are indicated. Negative fold changes denote gene repression for cells overproducing MqsA vs. the empty vector. IPTG (1 mM) was added to the empty plasmids (pBS[Kan] and pCA24N) and used to induce expression of MqsA from pBS[Kan]-mqsA and pCA24N-mqsA.
is the fragment that corresponds to the N-terminus of the coding sequence transcription factors that individually or synergistically respond to into the regulation of curli, responses in mice. Given that curli amyloids (and other extracellular SCIENTIFIC REPORTS notoriously implicated in chronic neurodegenerative disorders such neuronal stress. Under stressful conditions, however, MqsA is degraded by proteases, and MqsR is activated. MqsA degradation leads to derepression of rpoS and csgD, inhibition of fihD, and subsequently, results in increased biofilm formation. Therefore, this global regulative behavior of MqsA cements the role of antitoxins as far more than regulators of their own loci.

Methods

Bacterial strains, plasmids, and culture conditions. All strains and plasmids used in this study are summarized in Table 1. All strains were grown in lysogenic broth (LB, 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl), unless specifically indicated. Strains MG1655 ΔflhDΔrpoS and MG1655 ΔflhDΔrpoS (Table 1) were cultured using 50 µg/mL kanamycin and 5 µg/mL tetracycline. Kanamycin (50 µg/mL) was also used to maintain pBS(Kan)-based plasmids and to select for E. coli/pMCS2133ΔcsgD. Chloramphenicol (30 µg/mL) was used to maintain pCA24N-based plasmids. The CmqsRA-f and CmqsRA-r primers (Table 2) were used to confirm the mqsRA deletion in BW25113 ΔmqsRA via PCR and DNA sequencing. Similarly, the rpoS-f and gynR-r primers (Table 2) were used to verify the rpoS deletion in BW25113 ΔrpoS via PCR and DNA sequencing. BW25113 ΔmqsRA Δkmr was created by eliminating the KmR cassette in BW25113 ΔmqsRA Δkmr using FLP recombination encoded by pCP200.

Congo red (CR) assay. Curli production was examined by CR-binding assay at 28 °C using agar plates and planktonic cells. Two µL of each overnight culture was spotted on salt-free CR plates (10 g/L tryptone, 5 g/L yeast extract, 1 mM IPTG, 40 µg/mL CR, 20 µg/mL Coomassie Blue). Plates were incubated for 7 days, and the appearance of red colonies indicates binding to CR. Quantification of CR-binding was performed by measuring the amount of CR binding in planktonic cells. Briefly, 1 mL of each overnight culture was harvested at 13,000 X g centrifugation for 2 min, and washed with 1 mL of T-broth (10 g/L tryptone). Cells were resuspended in T-broth containing 1 mM IPTG and 40 µg/mL CR, and were incubated at 28°C for 2 to 24 h. Prior to incubation, an aliquot of cell suspension was removed, and CR was measured spectrophotometrically at 490 nm (Abs490). At specified time points, cells were harvested at 13,000 X g centrifugation for 10 min, and the supernatant was spectrophotometrically measured at 490 nm (Abs490 unbound). The Abs490 bound was therefore calculated from Abs490 unbound - Abs490 unbound and the amount of CR bound to cells (µg) was obtained from a standard curve constructed using 0 to 40 µg of CR dissolved in T-broth. All CR values were normalized using cell densities (OD600).

Scanning electron microscopy (SEM). Curli formation in cells in colony biofilms was determined using two-day old colonies grown on salt-free LB agar with 1 mM IPTG at 28 °C using agar plates and planktonic cells. Two µL of each overnight culture was spotted on salt-free CR plates (10 g/L tryptone, 5 g/L yeast extract, 1 mM IPTG, 40 µg/mL CR, 20 µg/mL Coomassie Blue). Plates were incubated for 7 days, and the appearance of red colonies indicates binding to CR. Quantification of CR-binding was performed by measuring the amount of CR binding in planktonic cells. Briefly, 1 mL of each overnight culture was harvested at 13,000 X g centrifugation for 2 min, and washed with 1 mL of T-broth (10 g/L tryptone). Cells were resuspended in T-broth containing 1 mM IPTG and 40 µg/mL CR, and were incubated at 28°C for 2 to 24 h. Prior to incubation, an aliquot of cell suspension was removed, and CR was measured spectrophotometrically at 490 nm (Abs490). At specified time points, cells were harvested at 13,000 X g centrifugation for 10 min, and the supernatant was spectrophotometrically measured at 490 nm (Abs490 unbound). The Abs490 bound was therefore calculated from Abs490 unbound - Abs490 unbound and the amount of CR bound to cells (µg) was obtained from a standard curve constructed using 0 to 40 µg of CR dissolved in T-broth. All CR values were normalized using cell densities (OD600).

RfRNA isolation and qRT-PCR. Total RNA was isolated from planktonic cells using a Qiagen RNeasy Mini kit, as described previously. qRT-PCR (1 mM) was used to produce MqsA in planktonic cells (IPTG was also added to the empty plasmid controls) for various durations and in various growth medium at 28°C (Table 3). RNA integrity was checked by agarose gel electrophoresis, and by the RNA260/RNA280 ratio of 1.85 to 2.25. qRT-PCR was performed according to manufacturer’s instructions (Power SYBR Green RNA-to-ct, 1-Step kit, Life Technologies, Carlsbad, SEM (FEI NanoSEM 630 FESEM) operating under low vacuum mode (5 kV).
Electrophoretic mobility shift assay (EMSA). Gene promoters were PCR-amplified from the genomic DNA of E. coli BW25113 and 3’-labelled with biotin (Biotin 3’-end DNA Labeling kit, Thermo Scientific, Walham, MA). Primers p-mqsA-f and p-mqsA-r (Table 2) were used to amplify p-mqsA (corresponds to position 3166514 bp to 3166774 bp of E. coli MG1655; Genbank accession number: U00096.2). Primers EMSA-cqsD-f and EMSA-cqsD-r (Table 2) were used to amplify p-cqsD (corresponds to position 1102383 bp to 1102694 bp of E. coli MG1655). The mqsA-N fragment (that codes for the 253 bp at the 5’ coding sequence of mqsA) was generated by primers mqsA-f and mqsA-N-r (Table 2). To investigate MqsA binding specificity, complimentary oligonucleotides with their 3’ end labelled with biotin were synthesized, solubilized, and annealed as previously described31. Palindrome 1 of p-mqsRA is at position 3166595 bp to 3166609 bp; palindrome 2 is at position 3166629 bp to 3166639 bp; Genbank accession number: U00096.2.

Binding reactions were performed in 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM DTT and 1 µg of poly (dl.dC). Briefly, 30 to 50 fmol of labeled DNA probe was incubated with purified MqsA in excess at ambient temperature for 1 h. Purified MqsA was sequentially obtained by His-tagged purification, removal of His6 tag by cleavage with TEV, and size exclusion chromatography32. The reactions were electrophoresed on a 5% polyacrylamide gel (Life Technologies, Carlsbad, CA) at 100 V in 0.5x TBE buffer for 90 min. Samples in the gel were electroblotted onto a nylon membrane (GE Healthcare, Little Chalfont, UK) at 380 mA in 1x TBE buffer for 1 h. The membrane was UV-crosslinked at 302 nm for 20 min, and detection was carried out using the protocol described in the Chemiluminescent Nucleic Acid Detection Module (Thermo Scientific, Walhам, MA).

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**Author contributions**

T.K.W. conceived the project, and V.W.C.S. performed the experiments. T.K.W. and V.W.C.S. analyzed the data and wrote the manuscript.

**Additional information**

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