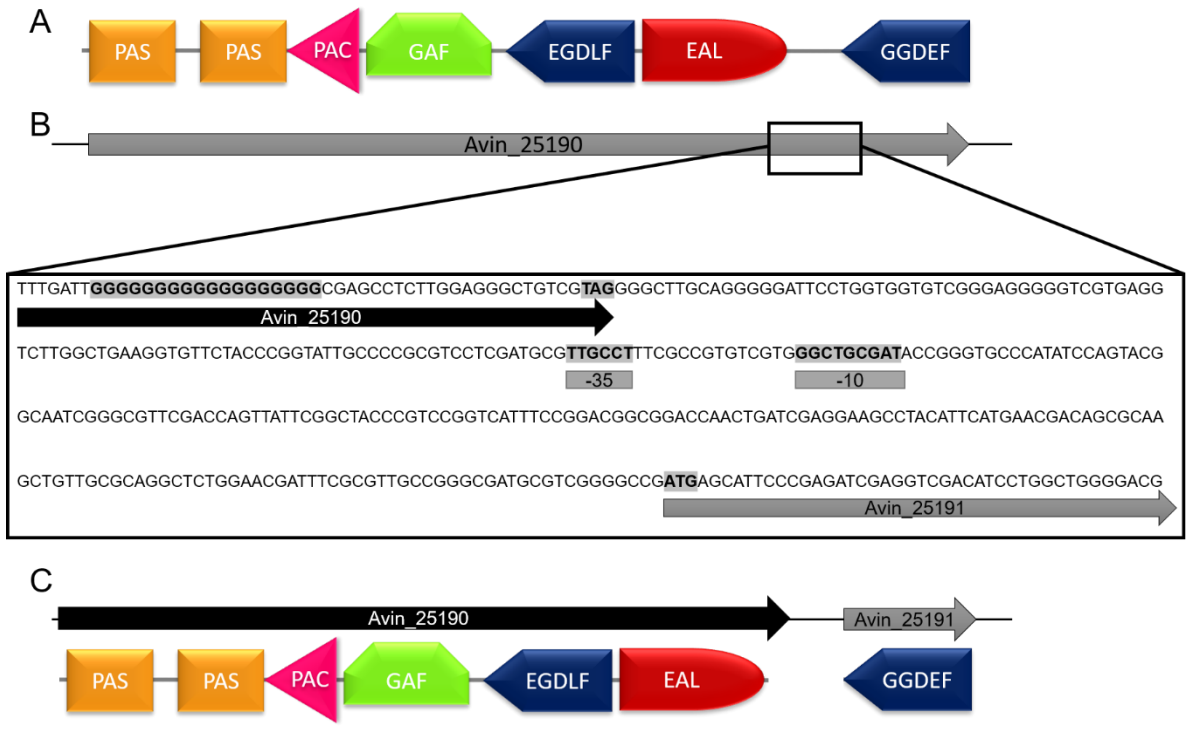

Figure S1. Analysis of the Avin_25190 *locus* of *A. vinelandii*. A. Predicted domain architecture of the annotated Avin_25190 encoded protein in the genome of strain DJ (NCBI genome, RefSeq: NC_012560.1). B. DNA sequence analysis of ORF Avin_25190. DNA sequence analysis of Avin_25190 in strain AEIV led us to identify two independent ORFs (named Avin_25190 and Avin_25191) having an intergenic region of 301 bp. The error in the original annotation in strain DJ was derived from a frameshift caused by the absence of a guanine residue in a poly-G region (highlighted in grey), right before the stop codon (TAG) of the first ORF. A predicted σ^{70} promoter driving transcription of Avin_25191 was identified, using the Soft Berry BPROM program (1). C. Domain architecture of the final Avin_25190 and Avin_25191 encoded proteins in the genome of strain AEIV.

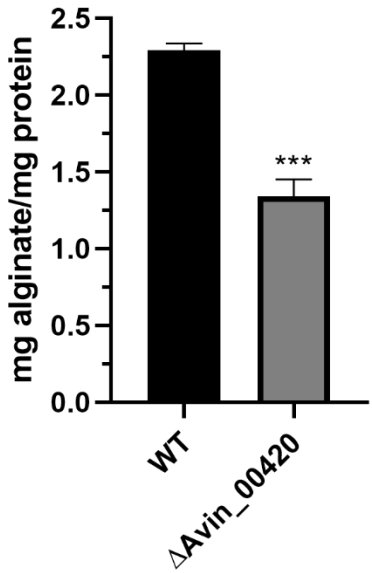

Figure S2. Alginate quantification in the ATCC 9046 *A. vinelandii* strain (black bar) and its derivative carrying a deletion of the Avin_00420 gene (ΔAvin_00420; grey bar), encoding the DGC *Av*GReg. Cells were cultured in Burk'-sucrose medium for 48 h. Means and standard deviation from three independent experiments are shown. Significant differences were analyzed by *t*-test. Statistical significance is indicated (***$p<0.001$).

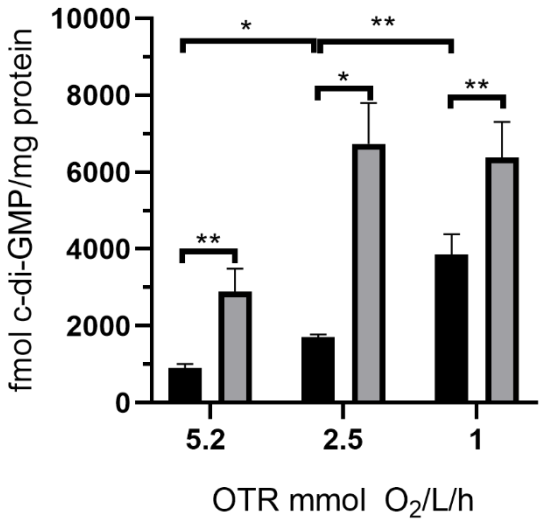

Figure S3. c-di-GMP quantification in *A. vinelandii* strains under different OTR_{max}. Quantification of c-di-GMP in the AEIV strain (black bars) and the Δ*mucG* mutant (grey bars) cultured in Burk'-sucrose medium for 48 h at the indicated OTR. Means and standard deviation from three independent experiments are shown. Significant differences were analyzed by *t*-test. Statistical significance is indicated (* $p<0.05$ or ** $p<0.01$).

Table S1. Strains and plasmids used in the present study.

Name	Genotype/Relevant characteristics	Reference
Strains		
AEIV (also named E strain)	Wild type strain	(2)
ATCC 9046	Wild type strain	ATCC collection
Escherichia coli DH5α	*recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1D* (*lacZYA-argF* U169 [φ80d*lacZDM15*] F- *Nalr*)	(3)
CLAM04	AEIV derivative in which the *mucG* gene was deleted and replaced by a Sp^r cassette (Δ*mucG*::Sp). Sp^r	This work
CLAM05	CLAM04 derivative, carries ORF Avin_50640 under the control of a σ^{70} promoter. Sp^r Tc^r	This work
CLAM01	AEIV derivative, carries a *mucG* allele with an EAL inactivated domain ($mucG^{AAA}$). Sp^r	(4)
CLAM06	AEIV derivative, carries a *mucG* allele with a GGDEF inactivated domain ($mucG^{GGAAF}$). Sp^r	This work
CLAM07	AEIV derivative, carries ORF Avin_00420 under the control of a σ^{70} promoter. Tc^r	This work
ICM01	AEIV derivative, carries a Δ*mucR*::Gm mutation	(5)
CLAM08	AEIV derivative in which the Avin_00370 gene was deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM09	AEIV derivative in which the Avin_00530 gene was deleted and replaced by a Km^r cassette. Km^r	This work
CLAM10	AEIV derivative in which the Avin_04950 gene was deleted and replaced by a Km^r cassette. Km^r	This work
CLAM11	AEIV derivative in which the Avin_05790 gene was deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM12	AEIV derivative in which the Avin_28640 gene was deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM13	AEIV derivative in which the Avin_37830 gene was deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM14	AEIV derivative in which the Avin_38420 gene was deleted and replaced by a Km^r cassette. Km^r	This work

CLAM15	AEIV derivative in which the Avin_39460 gene was deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM16	AEIV derivative in which the Avin_44470 gene was deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM17	AEIV derivative in which the Avin_00420 gene was deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM18	AEIV derivative in which the Avin_08240 gene was deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM19	AEIV derivative in which the Avin_11600 gene was partially deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM20	AEIV derivative in which the Avin_22780 gene was deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM21A	AEIV derivative in which the Avin_25190 gene was deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM21B	AEIV derivative in which the Avin_25191 gene was deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM22	AEIV derivative in which the Avin_29300 gene was partially deleted and replaced by a Km^r cassette. Km^r	This work
CLAM23	AEIV derivative in which the Avin_33420 gene was inactivated by insertion of a Gm^r cassette. Gm^r	This work
CLAM24	AEIV derivative in which the Avin_48220 gene was deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM25	AEIV derivative in which the Avin_48930 gene was partially deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM26	AEIV derivative in which the Avin_49060 gene was partially deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM27	AEIV derivative in which the Avin_51660 gene was partially deleted and replaced by a Km^r cassette. Km^r	This work
CLAM28	AEIV derivative in which the Avin_34930 gene was inactivated by a Gm^r cassette. Gm^r	This work
CLAM29	AEIV derivative in which the Avin_25160 gene was partially deleted and replaced by a Gm^r cassette. Gm^r	This work
CLAM30	AEIV derivative in which the Avin_50640 gene was partially deleted and replaced by a Gm^r cassette. Gm^r	This work
JGAT255	ATCC9046 derivative in which the Avin_00420 gene was partially deleted and replaced by a Gm^r cassette. Gm^r	This work
AED-*gusA*	AEIV derivative carries a chromosomal *algD–GusA* transcriptional fusion. Gm^r	(6)

Plasmids		
pJET1.2/Blunt	Used for subcloning PCR products. Ap^r	Thermo Scientific
pHP45Ω	Source of the Ω Sp^r cassette	(7)
pBSL98	Source of the Gm^r cassette	(8)
pBSL141	Source of the Km^r cassette	(8)
pUMATc	Plasmid used for integration of DNA into the *melA locus* of *A. vinelandii.* Tc^r	(9)
pJG09	pJET1.2/Blunt derivative carrying a 3.2 kb fragment containing *mucG* and its regulatory region. Ap^r	(4)
pLA05	pJG09 derivative, in which the *mucG* gene was deleted by inverse PCR and replaced by a Sp^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM04 (Δ*mucG::*Sp).	This work
pLA06	pJET1.2/Blunt derivative carrying a 1439 pb fragment containing the N-terminal portion of the $mucG^{GGAAF}$ allele.	This work
pJG103	pJET1.2/Blunt derivative carrying the entire *mucG locus* and a Sp^r cassette immediately downstream as a selection marker.	(4)
pLA06-Sp	pJG103 derivative carries the $mucG^{GGAAF}$ mutated allele. The wild type 4.3 kb *XhoI* fragment was replaced by that from pLA06 containing the D452A, E453A mutations. Ap^r, Sp^r. This plasmid, previously linearized with *ScaI* was used to construct mutant CLAM06.	This work
pLA07	pJET1.2/Blunt derivative carrying a 200 pb containing the σ^{70} promoter and the ATG codon of the Gm^r cassette. Ap^r	This work
pLA08	pJET1.2/Blunt derivative carrying a 1463 pb containing the Avin_00420 coding sequence except for its ATG translation start codon. Ap^r	This work
pLA09	pLA07 derivative carrying the Avin_00420 ORF under the control of a σ^{70} promoter ($P\sigma^{70}$-Avin_00420). Ap^r. The Avin_00420 gene was cloned as a *BamHI-XbaI* fragment from pLA08.	This work
pLA10	pUMATc derivative carrying the $P\sigma^{70}$-Avin_00420 construction from pLA09, released as a *BglII* fragment. Ap^r, Tc^r. This plasmid, linearized with *ScaI*, was used to construct strain CLAM07.	This work
pLA11	pJET1.2/Blunt derivative carrying a 1106 bp fragment containing ORF Avin_50640, except its ATG translation initiation codon. Ap^r	This work

pLA12	pLA07 derivative carries the Pσ^{70}-Avin_50640 construction. The Avin_50640 gene was cloned as a *BamHI-XbaI* fragment from pLA11. Ap^r	This work
pLA13	pUMATc derivative carrying the Pσ^{70}-Avin_50640 construction from pLA12 released as a *BlgII* fragment. Ap^r, Tc^r. This plasmid, linearized with *ScaI*, was used to construct strain CLAM05.	This work
pLA14	pJET1.2/Blunt derivative carrying a 2523 bp fragment containing ORF Avin_00370	This work
pLA15	pJET1.2/Blunt derivative carrying a 2789 bp fragment containing ORF Avin_00530	This work
pLA16	pJET1.2/Blunt derivative carrying a 1662 bp fragment containing ORF Avin_04950	This work
pLA17	pJET1.2/Blunt derivative carrying a 2909 bp fragment containing ORF Avin_05790	This work
pLA18	pJET1.2/Blunt derivative carrying a 3481 bp fragment containing ORF Avin_28640	This work
pLA19	pJET1.2/Blunt derivative carrying a 2917 bp fragment containing ORF Avin_37830	This work
pLA20	pJET1.2/Blunt derivative carrying a 1990 bp fragment containing ORF Avin_38420	This work
pLA21	pJET1.2/Blunt derivative carrying a 2333 bp fragment containing ORF Avin_39460	This work
pLA22	pJET1.2/Blunt derivative carrying a 2418 bp fragment containing ORF Avin_44470	This work
pLA23	pLA14 derivative, in which the Avin_00370 gene was deleted by inverse PCR and replaced by a Gm^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM08.	This work
pLA24	pLA15 derivative, in which the Avin_00530 gene was deleted by inverse PCR and replaced by a Km^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM09.	This work
pLA25	pLA16 derivative, in which the Avin_04950 gene was deleted by inverse PCR and replaced by a Gm^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM10.	This work
pLA26	pLA17 derivative, in which the Avin_05790 gene was deleted by inverse PCR and replaced by a Gm^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM11.	This work
pLA27	pLA18 derivative, in which the Avin_28640 gene was deleted by inverse PCR and replaced by a Gm^r cassette cloned into an	This work

	artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM12.	
pLA28	pLA19 derivative, in which the Avin_37830 gene was deleted by inverse PCR and replaced by a Gm^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM13.	This work
pLA29	pLA20 derivative, in which the Avin_38420 gene was deleted by inverse PCR and replaced by a Km^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM14.	This work
pLA30	pLA21 derivative, in which the Avin_39460 gene was deleted by inverse PCR and replaced by a Gm^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM15	This work
pLA31	pLA22 derivative, in which the Avin_44470 gene was deleted by inverse PCR and replaced by a Gm^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM16.	This work
pLA32	pJET1.2/Blunt derivative carrying a 2338 bp fragment containing ORF Avin_00420	This work
pLA33	pLA32 derivative, in which the Avin_00420 gene was deleted by inverse PCR and replaced by a Gm^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM17.	This work
pLA34	pJET1.2/Blunt derivative carrying a 2307 bp fragment containing ORF Avin_08240	This work
pLA35	pJET1.2/Blunt derivative carrying a 2307 bp fragment containing ORF Avin_11600	This work
pLA36	pJET1.2/Blunt derivative carrying a 1808 bp fragment containing ORF Avin_22780	This work
pLA37	pJET1.2/Blunt derivative carrying a 4683 bp fragment containing ORF Avin_25190 and Avin_25191	This work
pLA38A	pLA37 derivative, in which the Avin_25190 gene was deleted by inverse PCR and replaced by a Gm^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM21A.	This work
pLA38B	pLA37 derivative, in which a 2531 pb *Xho*I-*Stu*I fragment was deleted.	This work
pLA38C	pLA38B derivative, in which the Avin_25191 gene was deleted by inverse PCR and replaced by a Gm^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM21B.	This work

pLA39	pJET1.2/Blunt derivative carrying a 2033 bp fragment containing ORF Avin_29300	This work
pLA40	pJET1.2/Blunt derivative carrying a 1109 bp fragment containing ORF Avin_33420	This work
pLA41	pJET1.2/Blunt derivative carrying a 1946 bp fragment containing ORF Avin_48220	This work
pLA42	pLA41 derivative, in which the Avin_48220 gene was deleted by inverse PCR and replaced by a Gm^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM24.	This work
pLA43	pJET1.2/Blunt derivative carrying a 1783 bp fragment containing ORF Avin_48930	This work
pLA44	pJET1.2/Blunt derivative carrying a 2633 bp fragment containing ORF Avin_49060	This work
pLA45	pJET1.2/Blunt derivative carrying a 2740 bp fragment containing ORF Avin_51660	This work
pLA46	pJET1.2/Blunt derivative carrying a 769 bp fragment containing ORF Avin_34930	This work
pLA47	pJET1.2/Blunt derivative carrying a 2083bp fragment containing ORF Avin_25160	This work
pLA48	pJET1.2/Blunt derivative carrying a 2038 bp fragment containing ORF Avin_50640	This work
pLA49	pLA34 derivative, in which the Avin_08240 gene was partially deleted. A 1301 bp *Sal*I fragment was replaced by a Gm^r cassette. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM18.	This work
pLA50	pLA35 derivative, in which the Avin_11600 gene was partially deleted. A 1643 bp *Sph*I fragment was replaced by a Gm^r cassette. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM19.	This work
pLA51	pLA36 derivative, in which the Avin_22780 gene was deleted. A 1047 bp *Sal*I-*Stu*I fragment was replaced by a Gm^r cassette. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM20.	This work
pLA52	pLA39 derivative, in which the Avin_29300 gene was partially deleted. A 732 bp *Eco*RV-*Sph*I fragment was replaced by a Km^r cassette. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM22.	This work
pLA53	pLA40 derivative, in which the Avin_33420 gene was inactivated by insertion of a Gm^r cassette in a *Bgl*II restriction site. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM23.	This work

pLA54	pLA43 derivative, in which the Avin_48930 gene was partially deleted. A 954 bp *Apa*I-*Sph*I fragment was replaced by a Gm^r cassette. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM25	This work
pLA55	pLA44 derivative, in which the Avin_49060 gene was partially deleted. A 1510 bp S*tu*I-*Sph*I fragment was replaced by a Gm^r cassette. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM26.	This work
pLA56	pLA45 derivative, in which the Avin_51660 gene was partially deleted. A 924 bp *EcoR*I fragment was replaced by a Km^r cassette. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM27.	This work
pLA57	pLA46 derivative, in which the Avin_34930 gene was inactivated. A *EcoR*V restriction site was used to introduce a Gm^r cassette. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM28.	This work
pLA58	pLA47 derivative, in which the Avin_25160 gene was partially deleted. A 3449 bp *EcoR*I-*Nde*I fragment was replaced by a Gm^r cassette. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM29.	This work
pLA59	pLA48 derivative, in which the Avin_50640 gene was partially deleted. A 120 bp *Sma*I fragment was replaced by a Gm^r cassette. This plasmid, previously linearized with *ScaI*, was used to construct mutant CLAM30.	This work
pJGAT420	pJET1.2/Blunt derivative carrying a 2338 bp fragment containing ORF Avin_00420	This work
pJGAT420-Gm	pJGAT255 derivative, in which the Avin_00420 gene was deleted by inverse PCR and replaced by a Gm^r cassette cloned into an artificially generated *BamHI* site. This plasmid, previously linearized with *ScaI*, was used to construct mutant JGAT255.	This work

Table S2. Oligonucleotides used in the present study.

Primer Name	Nucleotide sequence (5′–3′)	Template	Used for
xseB1AhBm-F	GGACCGTTCGTCCGGATCCAGGG TCTGCCGGCGTTC	pJG09	*mucG* deletion by inverse PCR
mucGD-R	TGGATCCTCGGCTCAGCATTTGGG GTC		
GG9-RT-F	CAACCGCCACTATCTGGTC	pJG09	Amplification of a 224 bp fragment with the desired mutations (GAC → GCC and GAA → GCG) to construct the mucGGGAAF variant
GGDEF(GGAAF)-R	CCAGCAGCAGGAGGAACGCGGCG CCGCCGGAGCGGGCCACCAGC		
mucG-R	GATTGCTCGAAATCGACGGCG	pJG09	Amplification by overlapping PCR of the mucGGGAAF allele using the 224 bp fragment. The 1439 bp product was cloned producing pLA06 plasmid
pGm-F	TTTGCCCATGGACGCACAC	pSRK-Gm	Amplification of the promoter region of the Gm cassette contained in plasmid pLA07
pGmBh1-R2	AGGATCCCATCGTTGCTGCTCCAT AAC		
00420Bh1-F	TGGATCCTCTATGCCCGGTATCTC CC	Chromosomal DNA	Amplification of Avin_00420 contained in plasmid pLA08
00420-R	CAGAACGGCTGATCCACATCCC		
50640Bh1-F	TAGGATCCAAAGCCACCATCCTC GTCGTGG	Chromosomal DNA	Amplification of Avin_50640 contained in plasmid pLA11
50640-R	CATGTGGGCGCAGATCAAGG		
00370-F	CATCACGGCATGGTGATCG	Chromosomal DNA	Amplification of Avin_00370 contained in plasmid pLA14
00370-R	CGGATTCGAACCGGATGTG		
avin00370-FD	CGGATCCAAACTCTCGCCTACCG	pLA14	Avin_00370 deletion by inverse PCR
avin00370-RD2	TAGGGTCTCTGGGTAAAGTG		
avin00530-F	CGGCCTTGTTGGTGAAAGTC	Chromosomal DNA	Amplification of Avin_00530 contained in plasmid pLA15
avin00530-R	GGCTATTCGCTGTCCGAACG		
avin00530-RD	GGGATCCGTTCCAATTGACGCATC CAG	pLA15	Avin_00530 deletion by inverse PCR
avin00530-FD	GCGACGAGATCCAGGGTTAC		
Avin04950-F	GACATGGCACTTGCATGAC	Chromosomal DNA	Amplification of Avin_04950 contained in plasmid pLA16
Avin0490-R	AATAGGCGTACCGCTCGTC		
Avin_04950-RD	AGGATCCAGCAGTTTGAGGCGAT GGTG	pLA16	Avin_04950 deletion by inverse PCR
Avin_04950-FD	ACGAGGATGCCGAGCAGTTG		
Avin05790-F	CTTCCAGGGCACCTATGTCTG		

Avin05790-R	TCGAATACCTGGCGGACAAG	Chromosomal DNA	Amplification of Avin_05790 contained in plasmid pLA17
Avin05790-RD	CGAATCCTTCGGGACCGTTG	pLA17	Avin_05790 deletion by inverse PCR
Avin05790-FD	TGGATCCTCAGCGCTCCTGACCGAACAG		
28640-F	GCCGTCCAATCGACTAAAC	Chromosomal DNA	Amplification of Avin_28640 contained in plasmid pLA18
28640-R	GGCAGGTAGAGAAGGTTCC		
28640-RD	TTCCGCATGTCAGGCACCAC	pLA18	Avin_28640 deletion by inverse PCR
28640-FD	AGGATCCCTGCTCGAGTTCTCCACAAG		
37830-F	CGGTTCCTTCGACAAGTTC	Chromosomal DNA	Amplification of Avin_37830 contained in plasmid pLA19
37830-R	CACCTCGCTCTGCATGTAG		
37830-RD	AGGATCCACAGCAGTCCGAGCAGAAG	pLA19	Avin_37830 deletion by inverse PCR
37830-FD	TCCGCACCATCATCCAGTTG		
Avin38420-F	TGTCGTCTACACTCATGGTG	Chromosomal DNA	Amplification of Avin_38420 contained in plasmid pLA20
Avin_38420-R	GTACACGGCCAACCACTTTC		
Avin38420-RD	CATGACGAACCGCTGCAGCAACAG	pLA20	Avin_38420 deletion by inverse PCR
Avin38420-FD	TGGATCCCTCTCCAGCGAGCCAAGCACAA		
39460-F	GTCATTTGCGGGCTGCAAG	Chromosomal DNA	Amplification of Avin_39460 contained in plasmid pLA21
39460-R	CTCGTCGGTGACGAACAAGG		
39460-RD	CCCGGCATGGAAATGGAAC	pLA21	Avin_39460 deletion by inverse PCR
39460-FD	AGGATCCAGCGATCAGGCACTGTACC		
Avin44470-F	ACGGGATTCCTGGCCTATC	Chromosomal DNA	Amplification of Avin_44470 contained in plasmid pLA22
Avin44470-R	CTGCGCTATCACTGGCATTAC		
Avin44470-RD	TGGATCCGAACTGTACAGGTAGGCGATCAC	pLA22	Avin_44470 deletion by inverse PCR
Avin44470-FD	AAGGCCTGCTACCGGATCTTC		
00420-F	ACCAGGATCTGTCACTTG	Chromosomal DNA	Amplification of Avin_00420 contained in plasmid pLA32
00420-R2	ATCCAGTTGCTCGACATCC		
00420-RD	TCC ATT CTG CGG CCT GTT G	pLA32	Avin_00420 deletion by inverse PCR
00420-FD	ACG GAT CCA CCT GCA ACT GAC CGT GAG		

08240-F	AACTGCGCCTGTTGTTTC	Chromosomal DNA	Amplification of Avin_08240 contained in plasmid pLA34
08240-R	CGGTGGAATACCGCATAG		
11600-F	GGCCAAGCTGGAATCCATC	Chromosomal DNA	Amplification of Avin_11600 contained in plasmid pLA35
11600-R	ATCGAGCTTGCCGAGGTTG		
22780-F	CCCATGGCTACACCTACTC	Chromosomal DNA	Amplification of Avin_22780 contained in plasmid pLA36
22780-R2	TCTTTCCAGTCCGGTATG		
25190-F	TCCCGAATTGGACAAAGC	Chromosomal DNA	Amplification of Avin_25190 and Avin_25191 contained in plasmid pLA37
25190-R	CCAACTTCGCGACAGTTTC		
25190-RD	CATGCAGCGCGAATCACAG	pLA37	Avin_25190 deletion by inverse PCR
25190(A)-FD	ACGGATCCTCTTGGAGGGCTGTCGTAG		
25190-FD	AGGATCCACGGGAGTGACGAGTATG	pLA38B	Avin_25191 deletion by inverse PCR
25190-RD	CATGCAGCGCGAATCACAG		
29300-F	GCATGAATGCGCACATCG	Chromosomal DNA	Amplification of Avin_29300 contained in plasmid pLA39
29300-R	TGTACGGCCTGTTCAGGAG		
33420-F	CTCCAGGAGCCGGTATATG	Chromosomal DNA	Amplification of Avin_33420 contained in plasmid pLA40
33420-R	AAGTCTCGCTCGTCACTTC		
48220-F	ACGACCTGACTCAGTACC	Chromosomal DNA	Amplification of Avin_48220 contained in plasmid pLA41
48220-R	GACTATACCGGCTTCAAC		
48220-DR	TAAAGCTCCGCTCCGGACTG	pLA41	Avin_48220 deletion by inverse PCR
48220-DF	TGGATCCTCAGTTAGGGCGGAAGCTC		
48930-F	CCAGATCATGGCGCACTAC	Chromosomal DNA	Amplification of Avin_48930 contained in plasmid pLA43
48930-R	GGCGGGTATCGTTGTAGAC		
49060-F	CGCTGACCACGATCAAATG	Chromosomal DNA	Amplification of Avin_49060 contained in plasmid pLA44
49060-R	ATTGCCCGATCAGCGGGATCGAC		
51660-F	CGATCTCACGGTCATCAG	Chromosomal DNA	Amplification of Avin_51660 contained in plasmid pLA45
51660-R	GAGCAGCTCCTTGAGTTC		
34930-FBh	TGGATCCTTGCTGGCCCTGCTCGAGGG	Chromosomal DNA	Amplification of Avin_34930 contained in plasmid pLA46
34930-R	ACATTCTAGCCGCCGTGTCG		
25160-F	CTGAGCCAATTCCTGAAC	Chromosomal DNA	Amplification of Avin_25160 contained in plasmid pLA47
25160-R	CAGGTGTTTCTGGTACTC		

50640-F	ATCGCCCATACCCTCAAG	Chromosomal DNA	Amplification of Avin_50640 contained in plasmid pLA48
50640-R	CTGTTCGTCAGCCACTTG		
00420-F	ACCAGGATCTGTCACTTG	ATCC9046 Chromosomal DNA	Amplification of Avin_00420 contained in plasmid pJGAT255
00420-R2	ATCCAGTTGCTCGACATCC		
00420-RD	TCC ATT CTG CGG CCT GTT G	pJGAT255	Avin_00420 deletion by inverse PCR
00420-FD	ACG GAT CCA CCT GCA ACT GAC CGT GAG		
qgyrAL-F	CAA GCT GGC CCA TGA ACT GCT C		qRT-PCR assay
qgyrAL-R	TGG GAA TCC TGG TCG GCA TCA C		qRT-PCR assay
qPCR-mucG-F	TCG ACC GAA CCC GTT TCG AG		qRT-PCR assay
qPCR-mucG-R	GAT CAG GCG GTG GTA GAG AC		qRT-PCR assay

References

1. Solovyev V, Salamov A. 2011. Automatic Annotation of Microbial Genomes and Metagenomic Sequences, in Metagenomics and its Applications in Agriculture, Biomedicine and Environmental Studies (ed. Li, R. W.) (Nova Science Publishers), 61-78.

2. Larsen B, Haug A. 1971. Biosynthesis of alginate: Part I. Composition and structure of alginate produced by *Azotobacter vinelandii* (Lipman). Carbohydr Res 17:287–296.

3. Hanahan D. 1983. Studies on transformation of *Escherichia coli* with plasmids. J Mol Biol 166:557–580.

4. Ahumada-Manuel CL, Guzmán J, Peña C, Quiroz-Rocha E, Espín G, Núñez C. 2017. The signaling protein MucG negatively affects the production and the molecular mass of alginate in *Azotobacter vinelandii*. Appl Microbiol Biotechnol 101:1521–1534.

5. Martinez-Ortiz IC, Ahumada-Manuel CL, Hsueh B, Guzmán J, Moreno S, Espín G, Cocotl-Yañez M, Waters C, Núñez C. accepted. Cyclic-di-GMP-mediated regulation of extracellular mannuronan C-5 epimerases is essential for cyst formation in *Azotobacter vinelandii*. J Bacteriol.

6. Quiroz-Rocha E, Bonilla-Badía F, García-Aguilar V, López-Pliego L, Serrano-Román J, Cocotl-Yañez M, Guzmán J, Ahumada-Manuel CL, Muriel-Millán LF, Castañeda M, Espín G, Nuñez C. 2017. Two-component system CbrA/CbrB controls alginate production in *Azotobacter vinelandii*. Microbiology, 163:1105–1115.

7. Fellay R, Frey J, Krisch H. 1987. Interposon mutagenesis of soil and water bacteria: a family of DNA fragments designed for in vitro insertional mutagenesis of Gram-negative bacteria. Gene 52:147–154.

8. Alexeyev MF, Shokolenko IN, Croughan TP. 1995. Improved antibiotic-resistance gene cassettes and omega elements for *Escherichia coli* vector construction and *in vitro* deletion/insertion mutagenesis. Gene 160:63–67.

9. Cocotl-Yañez M, Moreno S, Encarnación S, López-Pliego L, Castañeda M, Espín G. 2014. A small heat-shock protein (Hsp20) regulated by RpoS is essential for cyst desiccation resistance in *Azotobacter vinelandii*. Microbiology, 160:479–487.