Gardiner *et al.* Supporting Material

Supplementary Materials and Methods

Mutagenesis of *varR* in *N. italica* R11

A *N. italica* R11 *varR* allelic replacement mutant strain, Δ*varR* was constructed using the Splicing by Overlap Extension PCR (SOE-PCR) strategy (Horton, 1995), coupled with bi-parental conjugation (Thoma, 2009) and homologous recombination of the SOE-PCR fragment with the genome of the recipient bacterium. The SOE-PCR strategy involved the amplification of two 1000 bp PCR products that targeted the *varR* gene; the varUpF/R primers (Table S2) targeted the first half of the *varR* gene ('1'), and the remaining *varR* gene sequence ('2') was amplified using the varDnF/R primers, with the deliberate exclusion of a 100 bp region from within the gene. A chloramphenicol resistance marker was separately amplified from the plasmid vector pBBR1 MCS (Kovach et al., 1995) using primers targeting the chloramphenicol acetyltransferase (*cat*) gene (Table S2). The varUpR and varRDnF primers include 5' overhang sequences that corresponds to the complement sequences of the primers used to amplify the *cat* gene (Table S2). A recombinant *varR* knockout fragment was constructed by 'PCR splicing' (Horton, 1995) the 5'overhangs on the '1' and '2' PCR products onto the 5` and 3` ends of the *cat* PCR product. Specifically, the PCR products termed '1', '2' and 'cat' were pooled in a SOE-PCR reaction that involved 5 PCR cycles without primers to align the fragments followed by addition of the primers varUpF and 'varDnR for 25 cycles to amplify the entire *varR* knockout fragment. Following PCR product purification (Zymo Research Corporation, USA), the knockout fragment was phosphorylated using T4 Polynucleotide Kinase (New England Biolabs Inc) and ligated into a broad host range suicide vector pKmobG11 (Katzen et al., 1999) that had been treated with BamH1, Large (Klenow) Fragment DNA Polymerase I and Antarctic Phosphatase (New England Biolabs Inc). The suicide vector containing the *varR* knockout cassette was then delivered to strain R11 via an *E. coli* ST18 donor strain using bi-parental conjugation (Thoma, 2009). Putative strain R11 Δ*varR* exconjugants were selected for on half-strength marine agar plates supplemented with chloramphenicol and 50 μg ml-1 of 5-bromo-4-chloro-3-indoxyl-b-D-glucuronide (x-gluc). Putative conjugants were confirmed by colony PCR using primers varUpF and FlankR (Table S2), which amplified the respective gene knockout construct and a region on the chromosome of strain R11 adjacent to the knockout construct.

Complementation of the *varR* gene replacement knockout mutant

The WT *varR* gene was amplified by PCR using primers varUpF and varDnR, which target regions 1000 bp upstream and 1000 bp downstream of the gene, respectively, and therefore include potential upstream promoter regions or downstream terminator regions. The purified PCR product was cloned into the SmaI restriction site of the broad-host range plasmid vector pBBR1 MCS-5 (Kovach et al., 1995) using standard procedures (Ausubel et al., 1999), and transferred to Δ*varR* via bi-parental conjugation with the donor strain *E. coli* ST18 (Thoma, 2009). Putative conjugants were selected for on half-strength marine agar plates containing gentamicin and chloramphenicol and verified by PCR using 'UpF' and a reverse primer that targeted the gentamycin resistance cassette on the plasmid vector pBBR1 MCS-5 (Kovach et al., 1995) (Table S2).

Quantitative mass spectrometry (LC MS/MS) with Isobaric Tags for Relative & Absolute Quantitation (iTRAQ™) labelling

Protein concentrations for the crude protein extracts obtained from the biofilm and planktonic cells were determined using the 2D Quant assay (GE Healthcare, UK) according to the manufacturer's protocol. To provide a qualitative assessment of the sample quality, the proteins were separated using SDS-PAGE on a Mini-PROTEAN® TGX™ Precast Gel (Bio-Rad, USA) and stained with SimplyBlue™ SafeStain (Invitrogen, USA). One hundred micrograms of each of the crude protein extracts were reduced and the cysteines blocked before trypsinization with 5 µg of sequencing grade porcine modified trypsin (Promega, Madison, USA). LC MS/MS analysis was conducted to test the efficiency of digestion before labelling.

The peptides were labelled with the iTRAQ™ reagents according to manufacturer's instructions (AB SCIEX, Foster City, USA). Briefly, the iTRAQ™ Reagent vials were dissolved in absolute ethanol and spun down, before addition of the four reagents to the respective samples. Following 2 h incubation at room temperature, the labelled peptide samples were combined as one set and unbound iTRAQ™ reagents, trypsin and SDS were removed by strong cation exchange (SCX) chromatography using an ICAT® Cation Exchange Cartridge using an Opti-Lynx cartridge holder (AB SCIEX, USA) and a syringe pump (KD Scientific, USA) at a flow rate of 10 ml h^{-1} according to the manufacturer's instructions. Desalting was performed with an Oasis HLB Plus Light Cartridge (Waters Corporation, USA) at an injection rate of 10 ml h^{-1} as per the manufacturer's directions. The

eluent was vacuum-dried and the peptide pellets dissolved in 0.05% HFBA (heptafluorobutyric acid)/1% formic acid.

The solubilized peptides were chromatographed twice to provide two technical replicates by online strong cation exchange (SCX) and nano-C18 LC using an Ultimate HPLC with a Switchos and Famos autosampler system (LC-Packings, Netherlands). Peptide samples were loaded onto a small SCX micro trap (~500 micron × 12 mm, Poros S10, AB SCIEX, USA) and were eluted sequentially with 5, 10, 15, 20, 25, 30, 40, 50, 100, 250, 500, and 1,000 mM ammonium acetate (20 µl). The initial unbound fraction and each salt step fraction were concentrated and desalted onto a micro-C18 pre-column (500 µm × 2 mm; Michrom Bioresources, USA) at 15 µl min^{-1}. After a 10 min wash the pre-column was switched (Valco 10 port valve, Dionex) in line with a fritless nano-analytical column (75 µm × ~10 cm) containing C18 reverse phase media (5 µm, 200 Å, Magic, Michrom Bioresources, USA) eluted with a linear gradient of ACN (acetonitrile) in 0.1% (v/v) formic acid from 2% to 36% ACN for 74 min, followed by 1 min at 90% ACN at ~300 nl/min. The column was connected via a fused silica capillary to a low-volume tee (Upchurch Scientific, USA) where high voltage (2,300 V) was applied and the column tip was positioned ~1 cm from the orifice of a QStar Elite mass spectrometer (AB SCIEX, USA). The QStar was operated in information-dependent acquisition mode. A time-of-flight (TOF) MS survey scan was acquired (m/z 350-1750). The three most abundant multiply charged ions (counts > 25) were sequentially selected by the quadrupole for TOF MS/MS analysis. Tandem mass spectra were accumulated for up to 2.5 s (m/z 65-2,000) using the Advanced IDA parameters automatic MS/MS accumulation (set to 20) and automatic collision energy.

The search parameters in the ProteinPilot™ 3.0 software (AB SCIEX, USA) were set to: sample type = iTRAQ™ 4-plex (peptide labelled); cysteine alkylation = iodoacetamide; digestion = trypsin; identification focus = biological modifications; search effort = thorough ID. To correct for small differences in protein loading, the generated ratios of all detected proteins were normalized by bias correction with the ProteinPilot™ software.

Table S1: Bacterial strains and plasmids used in this study

Strain or plasmid	**Relevant genotype**	**Reference**
***N. italica* R11**		
WT	Wild type strain	Case et al. (2011)
Δ*varR*	*varR* mutant: Δ*varR*::*Cat Kan*	This study
CΔ*varR*	Complemented *varR* mutant; Δ*varR*::*Cat Kan*; pBBR1-varR_wt, *Gen*	This study
E. coli		
E. coli DH5-α	supE44 *hsdR17 recA1 endA1 gyrA96* thi-1	
ST18	Δ*hemA* TpR *recA*, thi, pro, *hsdR*-M+RP4: 2-Tc:Mu: *Kan* Tn7 *λpir*	Thoma (2009)
Plasmids		
pKmobGII	5.9 kb broad host range mobilizable suicide vector, R6K *ori mob gusA Kan*	Katzen et al. (1999)
pKmobGII varR	Knock-out construct of *varR Kan Cat*	This study
pBBR1 MCS5	4.72 kb broad host range mobilizable vector, *rep mob Gen*	Kovach et al. (1995)
pBBR1-varR_wt	Complementation vector containing intact *varR*, *Gen*	This study

#*Cat*, chloramphenicol resistance; *Kan*, kanamycin resistance; *Gen*, gentamycin resistance; *λ pir* (a lambda prophage *pir* gene), π protein for plasmid replication of the R6K *ori*; *gusA*, beta-glucuronidase

Table S2: Primers used in this study to generate and confirm the *N. italica* R11 strains, Δ*varR* and C Δ*varR*. The 5' overhang sequences on the 'UpR' and 'DnF' primers that align with the CmF and CmR primers are given in italics.

PCR product	**Primer pair**	**Sequence 5'- 3'**	**Amplicon size (bp)**
cat	CmF/CmR	GCTGCATTAATGAATCGGCCA/ GAATAAATACCTGTGACGGAAGATCACTTC	903
'1'	varUpF/ varUpR	ATCGCCTTTCAAACCAATCT/ *TGGCCGATTCATTAATGCAGC*CATCACCTCGAAGACCAACC	1090
'2'	varDnF/ varDnR	*GAAGTGATCTTCCGTCACAGGTAATTATTC*G CCTTTAGTCCGTGGTTCAG/ CAACCGCATTCCAAGTAACC	923
varR knockout fragment	varUpF/ varRDnR	ATCGCCTTTCAAACCAATCT/ CAACCGCATTCCAAGTAACC	2916
Δ*varR* confirmation	varUpF/ FlankR	ATCGCCTTTCAAACCAATCT/ CATCCAGTGTTTTGGGCTTT	3025
CΔ*varR* confirmation	GenF/ varDnF	GACGCACACCGTGGAAA/ GAAGTGATCTTCCGTCACAGGTAATTATTCGCCTTTAGTCCGTGGTTCAG	2238

Supplementary Results

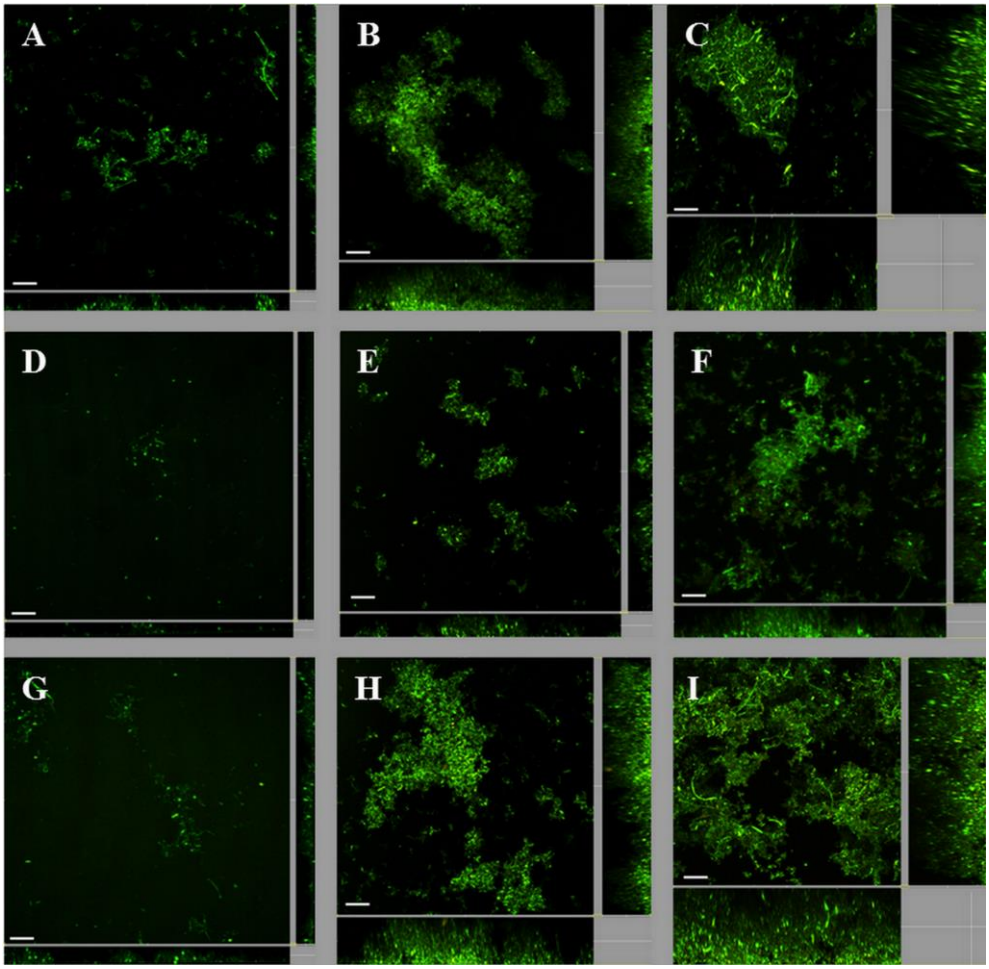

Figure S1: Confocal Microscopy images showing typical biofilm formation for *N. italica* R11 WT (A B C), Δ*varR* (D E F), and CΔ*varR* (G H I) after 24 h (A D G), 48 h (B E H), and 72 h (C F I) growth. Bacteria are stained with LIVE/DEAD® BacLight™ (Molecular Probes): live cells are fluorescing green; dead cells are red; cells that are in the process of dying are yellow. Z-stack images were acquired using an Olympus Fluoview FV1000 Confocal Laser Scanning Microscope and processed using IMARIS software. Scale bar = 20µm

Table S3: Proteins that were significantly differentially expressed in *N. italica* R11 WT cells under biofilm growth (WTB) compared to planktonic conditions (WTP). Proteins were detected with $p < 0.05$ across two biological replicates, except where * denotes the protein was identified in all three biological replicates. Shading denotes proteins that were down regulated in expression in WTB relative to WTP. The Genbank accession number for the proteins is given along with the protein description and COG category as determined in the NCBI database.

Accession number	NCBI Protein Annotation	Fold change in expression
	C: Energy production and conversion	
EEB72409	Malate synthase G	6.9
EEB71438	Aconitrate hydratase 1	4.9
EEB70986	Pyruvate carboxylase	2
EEB72091	2-oxoglutarate dehydrogenase, E1 component	-2.3
EEB71804	Indolepyruvate ferredoxin oxidoreductase	-2.4
EEB72637	Flavodoxin oxidoreductase, gamma subunit protein	-2.6*
EEB72898	Phenylacetic acid degradation protein, PaaN	-3.3
EEB72553	Proline dehydrogenase family protein	-5.4*
EEB72453	NADH-quinone oxidoreductase, G subunit	-5.4
EEB71249	Aldehyde dehydrogenase family protein	-5.6*
EEB69852	Aconitrate hydratase B	-5.7*
EEB70645	Methylmalonic acid dehydrogenase	-7.3*
	E: Amino acid transport and metabolism	
EEB72454	Peptidase family M3 protein	7.3*
EEB70871	Aminotransferase class IV	7.1
EEB70400	Ornithine cyclodeaminase	6.1*
EEB69952	Arginase	5.9*
EEB70113	ABC-type glycine transporter, substrate-binding	4.8*
EEB72013	ABC-type peptide transporter, substrate-binding	4.5*
EEB71507	Gamma-glutamyltranspeptidase	4.2*
EEB69826	Peptidase family M20 protein	3.7
EEB70711	L-threonine 3-dehydrogenase	3.6*
EEB72079	Leucyl aminopeptidase	3.6*
EEB72156	Oligoendopeptidase F	3.5
EEB71087	Extracellular solute-binding proteins, family 5	3.5
EEB71181	Peptidase T	3.4
EEB69706	Aspartate-semialdehyde dehydrogenase	3
EEB69622	Glutamine synthetase, type I	2.9*
EEB72235	ABC-type oligopeptide transporter, periplasmic component	2.8
EEB71218	Cystathionine gamma-synthase like enzyme	2.3
EEB71049	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	2.2*

EEB72864	2-isopropylmalate synthase	-2.1
EEB71458	ABC-type putrescine transporter, periplasmic component	-2.3
EEB70780	ABC-type amino acid transporter, periplasmic component	-2.6
EEB72886	3-phosphoglycerate dehydrogenase	-2.9*
EEB72547	Twin-arginine translocation pathway signal	-3.2*
EEB70354	Extracellular solute-binding proteins, family 5	-4.7
EEB69263	Extracellular ligand-binding receptor	-7.9
	F: Nucleotide transport and metabolism	
EEB71027	5'-nucleotidase	3.5*
EEB72523	Phosphoribosyl transferase domain protein	-4.1
	G: Carbohydrate transport and metabolism	
EEB72081	Extracellular solute-binding protein, family 1	2.7*
EEB69962	HAD-superfamily subfamily IIA hydrolase	2.6
EEB70292	Triose-phosphate isomerase	2.4
EEB70779	ABC-type sugar transporter, ATPase component	-2.4
EEB69824	Extracellular solute-binding protein, family 1	-5.4*
EEB69330	Extracellular solute-binding protein, family 7	-6.7
	H: Coenzyme transport and metabolism	
EEB72422	S-adenosylmethionine synthetase	-2.7*
EEB72820	Aminotransferase class III	-2.2
EEB71985	Aminotransferase class III	-8.6
	I: Lipid metabolism and transport	
EEB71107	Polyhydroxyalkanoate depolymerase, intracellular	5.3
EEB71688	3-hydroxyisobutyrate dehydrogenase	3.1
EEB71680	Acetyl-CoA acetyltransferase	2.1
EEB70651	Acetoacetyl-CoA reductase	1.9
EEB72443	Methylmalonyl-CoA mutase	-2.7
EEB72740	Acetyl-CoA carboxylase, carboxyltransferase component	-3.2
EEB72812	Acyl-CoA dehydrogenase	-3.9
EEB72289	Acetyl/propionyl-CoA carboxylase, alpha subunit	-4.6
	J: Translation, ribosomal structure and biogenesis	
EEB71968	AsnC family transcriptional regulator	6.5
EEB72503	Glutathione synthetase	3.2
EEB69693	Alanyl-tRNA synthase	1.9
EEB71499	Translation elongation factor Ts	-2.6
EEB70221	Ribosomal protein L7/L12	-3.5
EEB72731	Ribosomal protein S10	-3.8
EEB72466	Translation elongation factor G	-4.2*
EEB71716	Ribosome-associated protein Y	-4.3
EEB69437	Ribosomal protein L3	-4.7
EEB72506	Ribosomal protein S2	-5.6
EEB72106	Ribosomal protein S1	-5.7
EEB71543	Ribosomal protein L15	-6.3*

EEB72511	Ribosomal protein L9	-6.7
EEB70057	Ribosomal protein L1	-7.3*
EEB72021	Ribosomal protein L6	-9
	K: Transcription	
EEB72887	Putative cold-shock DNA-binding domain protein	-3.5
EEB69671	MarR family transcriptional regulator	-11.4
	M: Cell wall/membrane biogenesis	
EEB70840	Outer Membrane Protein, OmpA	14.3
EEB70168	ABC-type/ RND efflux pump, membrane efflux protein	6.5*
EEB72598	Glutamine-fructose-6-phosphate transaminase	4.0*
EEB70281	Lytic murein transglycosylase	2.1
EEB70816	Outer membrane protein assembly complex, YaeT protein	-3.7
	N: Cell motility/ T: Signal transduction mechanisms	
EEB71824	Methyl-accepting chemotaxis sensory transducer	-6.9*
EEB69311	Histidine kinase	-9.8*
EEB69630	Methyl-accepting chemotaxis sensory transducer	-11.1*
	O: Posttranslational modification, protein turnover, chaperones	
EEB70350	DsbA oxidoreductase	9.8
EEB71705	Periplasmic serine protease, DO/DeqQ family protein	9.6*
EEB70501	Peroxiredoxin	9.6
EEB72280	Thiol-disulfide interchange protein, DsbA family	9.4*
EEB71775	FeS assembly protein SufB	5.2
EEB72892	ATP-dependent protease La	3.9*
EEB70461	ATP-dependent metallopeptidase, HflB subfamily	2.8*
EEB69723	ATP-dependent Clp protease, ATP-binding subunit	2.5
EEB72870	Chaperonin GroEL	1.6
	P: Inorganic ion transport and metabolism	
EEB72831	Superoxide dismutase	15.2*
EEB70756	ABC-type zinc/manganese/iron transporter, periplasmic	10.8*
EEB69747	Extracellular solute-binding protein, family 1	10.2
EEB69398	TonB-dependent receptor, plug	4.5*
EEB71350	Toxic anion resistance protein	2.9
EEB69252	ABC- type transporter, periplasmic hemin-binding protein	2.3
	Q: Secondary metabolites biosynthesis, transport and catabolism	
EEB69518	ABC-type organic solvent transporter, auxiliary component	12.4*
EEB71612	Extracellular solute-binding protein, family 7	3.1
	R: General function prediction only	
EEB70957	Imelysin-like peptidase	7.6
EEB69320	Alpha/beta hydrolase family	7.4
EEB72784	TRAP transporter solute receptor, TAXI family	7.3

EEB71080	D-beta-hydroxybutyrate dehydrogenase	6.3
EEB71038	Cobaltochelatase, CobS subunit	4.6*
EEB72449	Amidohydrolase family protein	3.7
EEB70327	Amidase	2.4
EEB69264	Predicted epimerase, PhzC/PhzF homolog	2.3
EEB71376	3-hydroxyacyl-CoA dehydrogenase, type II	-2.3
EEB72893	Predicted nucleoside-diphosphate-sugar epimerase	-4.5
EEB70264	Uncharacterized periplasmic binding protein domain	-7.2
	S: Function unknown	
EEB69472	Phasin family protein	25.6*
EEB71183	Hypothetical	21.7*
EEB70321	Predicted periplasmic protein	15.8*
EEB70310	Hypothetical	15.4
EEB70606	Predicted outer membrane protein	15.1*
EEB70121	Hypothetical	9.9
EEB70107	Hypothetical	7.9*
EEB69525	Hypothetical	7.6*
EEB70709	Predicted periplasmic lipoprotein	7.5
EEB72259	Universal stress protein family protein	4.5
EEB70593	Hypothetical	4
EEB72283	Hypothetical	3.4
	T: Signal transduction mechanisms	
EEB72578	Response regulator containing CheY-like receiver, AAA-type ATPase, and DNA-binding domains	4.2
EEB70997	Serine protein kinase, PrkA	3.0*
EEB69307	Anti-sigma-factor antagonist	2.8
EEB69994	GTP-binding protein, TypA/BipA	-1.9

Table S4: Proteins differentially expressed in Δ*varR* planktonic cells (ΔVP) compared to WT planktonic cells (WTP). The average fold change in expression in ΔVP is shown, with grey shading indicating down-regulation. Proteins were detected with $p < 0.05$ across two biological replicates except where * denotes that were proteins identified in three biological replicates. Shading denotes proteins that were down regulated in expression in ΔVP relative to WTP. Underlined accession numbers indicate that the protein was also differentially expressed in Δ*varR* relative to WT under biofilm growth conditions (Table S5) Genbank accession numbers are given along with the COG category and protein description provided in the NCBI database.

Accession number	NCBI Protein Annotation	Fold change in expression
	C: Energy production and conversion	
EEB69905	ATP synthase F1, alpha subunit	2.7
	E: Amino acid transport and metabolism	
EEB70922	Diaminopimelate/ornithine decarboxylase	3.1
EEB70354	Bacterial extracellular solute-binding proteins, family 5	-2.8*
EEB71221	Bacterial extracellular solute-binding protein, family 8	-2.3
	G: Carbohydrate transport and metabolism	
EEB69628	Oligo-1,6-glucosidase	3*
EEB70046	Alpha-glucosides-binding periplasmic protein	2.2*
EEB72540	Transketolase	1.7
	I: Lipid transport and metabolism	
EEB71680	Acetyl-CoA acetyltransferase	-6.3*
	J: Translation, ribosomal structure and biogenesis	
EEB72430	3' exoribonuclease family protein	3.6
EEB72106	Ribosomal protein S1	2.5
EEB72021	Ribosomal protein L6	2.1
	M: Cell wall/membrane/envelope biogenesis/ U: Intracellular trafficking, secretion, and vesicular transport	
EEB72298	Outer membrane efflux protein, TolC family	-2
	O: Posttranslational modification, protein turnover, chaperones	
EEB72892	ATP-dependent protease La	3.1
EEB70029	Membrane protease subunits, stomatin/prohibitin homolog	-2.4
	P: Inorganic ion transport and metabolism	
EEB69373	Putative heme degradation protein	4.5
EEB69747	Extracellular solute-binding protein, family 1	5
	S: Function unknown	
EEB69472	Phasin family protein	-8.1
	T: Signal transduction mechanisms	
EEB70997	Serine protein kinase, PrkA	-6.9

Table S5: Proteins differentially expressed in Δ*varR* biofilm cells (ΔVB) compared to WT biofilm cells (WTB). The average fold change in expression in ΔVB is shown, with grey shading indicating down-regulation. Proteins were detected with $p < 0.05$ across two biological replicates except where * denotes that were proteins identified in three biological replicates. Shading denotes proteins that were down regulated in expression in ΔVB relative to WTB. Genbank accession numbers are given along with the COG category and protein description provided in the NCBI database. The qualifier ^ denotes that a protein with the same accession number was differentially regulated in the WT biofilm proteome (Table S3). Underlined accession numbers indicate that the protein was also differentially expressed in Δ*varR* relative to WT under planktonic growth conditions (Table S4)

Accession number	NCBI Protein Annotation	Fold change in expression
	C: Energy production and conversion	
EEB69852^	Aconitate hydratase	4*
EEB70986^	Pyruvate carboxylase	-2
EEB72814	Citrate synthase I	-6.1*
	E: Amino acid transport and metabolism	
EEB72300	ABC-type amino acid transporter, periplasmic binding	2.5
EEB69952^	Arginase	-1.4
EEB72013^	ABC-type peptide transporter, substrate-binding	-1.4
EEB70210	Glutamate dehydrogenase 1	-1.9
EEB70952	ABC-type spermidine/putrescine transporter, ATPase	-2.6
	I: Lipid transport and metabolism	
EEB70651^	Acetoacetyl-CoA reductase	-3.1
EEB71680^	Acetyl-CoA acetyltransferase	-8.2
EEB71107^	Polyhydroxyalkanoate depolymerase, intracellular	-8.8
	J: Translation, ribosomal structure and biogenesis	
EEB72106^	Ribosomal protein S1	4.5
EEB71543^	Ribosomal protein L15	4.8*
EEB69437^	Ribosomal protein L3	5.1
	M: Cell wall/membrane/envelope biogenesis	
EEB70816^	Outer membrane protein assembly complex, YaeT protein	3.8
	M: Cell wall/membrane/envelope biogenesis/ U: Intracellular trafficking and secretion	
EEB72298	Outer membrane efflux protein, TolC family	1.7
	N: Cell motility/ T: Signal transduction mechanisms	
EEB69630^	Methyl-accepting chemotaxis sensory transducer	6.1
	O: Posttranslational modification, protein turnover, chaperones	
EEB70734	Bacterial trigger factor protein	2.9
	Q: Secondary metabolites biosynthesis, transport and catabolism	

EEB71180	Extracellular solute-binding protein, family 7	7.2
	R: General function prediction only	
EEB69264^	Predicted epimerase, PhzC/PhzF homolog	-2
EEB69320^	Alpha/beta hydrolase family	-3.4
	S: Function unknown	
EEB70321^	Predicted periplasmic protein	-2.7
EEB69472^	Phasin family protein	-17*
	T: Signal transduction mechanisms	
EEB70997^	Serine protein kinase, PrkA	-2.3*

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