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Swarm and swim motilities of *Salmonella enterica* serovar Typhimurium and role of osmoregulated periplasmic glucans

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Abstract

Background: *Salmonella enterica* serovar Typhimurium SL 1344 migrates on moist surfaces by swarming motility. S. *enterica* serovar Typhimurium synthesized osmoregulated periplasmic glucans (OPG) using opgGH bicistronic operon under low osmolarity conditions (<70 mMos Mol l^{-1}). OPG were not detected when cells were grown in swarm motility-promoting media which were typically iso- or hyperosmotic (>400 mMos Mol l^{-1}).

Findings: We observed that an opgGH-deletion mutant was defective in swarm motility. Swarm motility was complimented by a plasmid bearing a wild type copy of opgGH. Since synthesis of OPG is below the detection limits at medium osmolarity >400 mMos Mol l^{-1} the requirement of opgGH operon for swarm motility appears counter intuitive. We observed that in wild-type cells, transcripts of opgGH genes remained high even at 600 mMos Mol l^{-1}, the highest osmolarity at which swarm motility occurred. Truncated and in-frame deletion copies of opgGH (carrying deletions in transmembrane domains) as well as plasmid expressing catalytically dysfunctional active site of OpgH ($OpgH_{D346G, D348G}$) failed to restore swarm motility.

Conclusions: Thus full-length opgGH gene products were needed to support swarm motility even though no OPG synthesis was detected in swarm motility growth media. The requirement of OPG for swarm motility appears to be indirect, since in the opgGH mutant, several class-II and -III flagella regulatory genes were down-regulated specifically under swarm growth conditions. It is postulated that the lack of OPG results in cells incapable of transducing surrounding environmental stimuli, possibly due to increased transcript levels of cyclic di-GMP (secondary messenger) modulator gene (*ydiV*) in the opgGH mutant under swarm growth conditions.

Keywords: Food borne pathogens, flagella function, pathogen transport, *salmonella*

Background

The periplasmic compartment of microorganisms of the family *Enterobacteriaceae* is composed of polymers, either branched or cyclic, with glucose as the sole carbohydrate moiety and are known as osmoregulated periplasmic glucans (OPG) [1,2]. Large quantities of OPG are synthesized as the osmolarity of the surrounding medium decreases [3]. In *Escherichia coli, Salmonella enterica* serovar Typhimurium and *Shigella flexneri,* OPG synthesis is catalyzed by the products of *opgG* and *opgH* genes, which are transcribed as a single operon [2,4-6]. Studies in several microorganisms, including plant and animal pathogens as well as plant symbionts, have shown the importance of OPG in successful host invasion [6-8]. OPG synthesis-deficient mutants result in compromised virulence in plant- and animal-pathogenic microorganisms [6-8]. In symbiotic plant-microbe interactions, mutant cells fail to gain entry in plant roots [9,10]. OPG of plant

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pathogenic bacterium, *Dickeya dadantii*, which causes soft-rot disease, appear to regulate two-component phosphorelay system, namely, RcsCD and RcsB, in a concentration dependent manner [7,11]. Possible dysfunction of phosphorelay pathway, coupled with pleiotropic effects of OPG mutants, suggest that lack of OPG synthesis renders cells incapable of judging the surrounding environment.

In *S. enterica* Serovar Typhimurium, mutations in *opgGH* operon result in compromised virulence [6,12]. Lack of OPG synthesis also show pleiotropic phenotypes such as extended lag time to enter a logarithmic growth phase as well as reduced swim-motility in low nutrient-low osmolarity media (with osmolarity <100 mMos Mol^{-1}). However, normal swim motility and growth were observed in isomolar growth media such as LB broth (osmolarity ca. 420 mMos Mol^{-1}), indicating specific role for OPG under hypo-osmotic growth conditions [6,12].

OPG have also been reported to be required for swarm motility phenotypes of *E. coli* [13]. A genome-wide mutation screen of *S. enteric* serovar Typhimurium also identified an *opgGH* requirement for swarm motility [14]. However, a role for OPG in swarm motility has not been investigated. Given the fact that virtually no OPG synthesis is reported when bacteria are grown on media with osmolarity >400 mMos Mol^{-1} [3-6], the requirement of OPG for swarm motility of *S. enterica* serovar Typhimurium appears paradoxical. Most swarm media reported for *Salmonella* have osmolarity >400 mMos Mol^{-1} [15,16]. Typically, swarm motility is observed under growth conditions of nutrient abundance and isomolar growth conditions such as LB semisolid media (0.5-0.6% agar) in presence of glucose (ca. 420 mMos Mol^{-1}) [13,14,16]. It has been documented in *E. coli, S. flexneri* and *Salmonella* sp. that synthesis of OPG is inversely proportional to the medium osmolarity, with maximum OPG synthesis occurring around 95 mMos Mol^{-1} (i.e., LB broth without NaCl) [3-6].

In order to examine the alleged role of OPG in swarming motility, we monitored *opgGH* transcripts from cells grown in liquid and swarm growth media of varying osmolarities. We also compared transcript levels of class I, II and III flagella gene regulators. Finally, we examined the possibility that the *opgH* gene product, an 847-amino acid protein with 8 transmembrane segments and 3 large cytoplasmic regions [17], may support swarm motility either by merely providing membrane-structural support (i.e., without catalytic function) by some unknown function(s) under conditions in which OPG synthesis is not believed to occur.

Methods

Bacterial strains and culture conditions

Salmonella enterica serovar strain SL1344 and its *opgGH* mutant strain [6] were streaked on LB agar plates from freezer stocks, and a single colony was inoculated in LB broth and grown at 37°C in a shaker incubator for 18-20 h. The medium was supplemented as needed with antibiotics at the following concentrations: ampicillin (100 µg ml^{-1}), kanamycin (50 µg ml^{-1}), nalidixic acid (10 µg ml^{-1}). Osmolarity of growth media was measured with Wescor vapor pressure osmometer (model 5500, Wescor, Inc., Logan UT).

opgG and opgH plasmid constructs

Plasmid construct pBK16 carrying wild-type gene copies of *opgG* and *opgH* was subjected to various restriction enzyme treatments to yield truncated genes of varying length (**Table 1**). Plasmid constructs were transfected into an *opgGH* mutant strain by electroporation for complementation studies as described earlier (Bhagwat et al., 2006). Site-directed mutagenesis at the active site residues of OpgH was carried out using a unique site elimination protocol [18] and mismatch repair deficient *E. coli* strain BMH 71-18 kit (Clontech, Mountain View, CA). In order to facilitate the cloning, 1.7 kb portion (EcoRV-NdeI fragment) of *opgGH* insert from pBK16 was cloned into pPQSL2.0 to yield pAAB11. Mutagenic and selection primers were 5'ggtggtgctgggcgcgggctcagtgatgag and 5'ccagtatacactcctctagagctgaggtctgc, respectively (altered nucleotide bases are in bold with underline). The mutagenic primer converted ^{346}Asp to ^{346}Gly and ^{348}Asp to ^{348}Gly (gac to ggc) while the selection primer converted a unique *NheI* site to an *XbaI* site (gctagc to tctaga) in the non-coding region of pPQSL2.0. The insert fragments with altered DNA sequences were subjected to *NheI* digestion (to eliminate wild-type sequences) and then cloned to yield pAAB12. The cloned DNA was confirmed to have lost the *NheI* site and gained an *XbaI* site by restriction digestion and standard agarose gel electrophoresis. Finally, the 1.7 kb *EcoRV-NdeI* insert from pAAB12, containing the site-directed mutations, was placed into pBK16 to yield pAAB13. Site directed mutations were confirmed by sequencing DNA from both strands. The pAAB13 and other truncated clones of pBK16 (i.e., pMD 258-264) were electroporated in the scarGH mutant and were examined for swim and swarm phenotypic complementation (**Table 1**).

Determination of swarming ability of *Salmonella*

All media were prepared using deionized water (DI; Milli-Q). Swarm agar plates were prepared by supplementing LB broth with 0.6% (wt/vol) agar and D-glucose was added (5 g/liter, filter sterilized and added separately) prior to pouring the plates. Overnight grown shake cultures of *S. enteric* serovar Typhimurium SL1344 were placed (5 µl) on swarm agar surface and allowed to dry for 30 min at room temperature. Swarm plates were incubated at 37°C for 10 h and swarm diameters were measured.

Swim motility was measured using 0.3% LB agar as described earlier [19].

Preparation of samples for transmission electron microscopy

Samples were prepared as described before [20]. Briefly, cells from swarm edges from LB swarm plates were fixed with equal amounts (v/v) of 2.5% glutaraldehyde and placed on formvar

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Table 1. Recombinant constructs of opgGH and their ability to compliment swim and swarm motility.

Name	Plasmid construction[a]	Comments	Complementation of swim (low osmolarity medium) or swarm motility of *opgGH* mutant[b]
pBK16	OpgG (517aa) OpgH (847 aa)	Wild-type	+
pMD264	OpgG* (313aa, BmgBI) OpgH (847aa)	In-frame deletion of amino acids 49-253 of OpgG	-
pMD263	OpgG (517aa) ZraI/MscI) OpgH* (279aa, ZraI/MscI)	Deletion of amino acids 280-847 of OpgH	-
pMD258	OpgG (517aa) OpgH* (290aa, EcoRV/MscI)	Deletion of amino acids 291-847 of OpgH	-
pMD262	OpgG (517aa) OpgH* (334aa, BglI)	Deletion of amino acids 335-847 of OpgH	-
pMD259	OpgG (517aa) OpgH* (468aa, FspI/MscI)	Deletion of amino acids 469-847 of OpgH	-
pMD261	OpgG (517aa) OpgH* (507aa, DraIII)	Deletion of amino acids 508-847 of OpgH	-
pMD260	OpgG (517aa) OpgH* (537aa, PstI)	Deletion of amino acids 538-847 of OpgH	-
pAAB13	OpgG (517aa) OpgH* (847aa)	Alterations at active site residues of OpgH ^{346}Asp to ^{346}Gly and ^{348}Asp to ^{348}Gly.	-

[a]pBK16 was digested and re-ligated with the indicated restriction enzyme generating truncated OpgGH genes. Construct was confirmed by restriction endonuclease digestion and was electroporated into GH mutant to check swim and swarm phenotypes.
[b]Swim phenotype was tested in low osmolarity low nutrient LB media. A construct was considered to be complimenting swim or swarm function if motility diameters >15 mm.

coated Ni grids for 5 min. After wicking excess fluid from the grid, bacteria were stained with 2% uranyl acetate for 2 min. The grids were examined with a Hitachi HT-7700 transmission electron microscope coupled to a bottom-mounted Advanced Microscopy Techniques XR-41C digital camera.

RNA extraction and quantitative PCR for determining gene expression

Salmonella cells from LB broth with varying amounts of NaCl were harvested in RNA stabilization reagent [21] and processed for RNA isolation as described [22]. Further, 0.5 µg RNA was used to make cDNA using random primer cDNA synthesis kit (New England BioLabs, MA) and quantitative PCR was performed using EvaGreen indicator dye followed by melting curve as described before [19]. PCR reactions were carried out in triplicate and three RNA preparations were used for each experiment. In order to determine relative transcription levels of individual genes, C_t values were normalized to dnaC transcripts within samples and processed using the formula $2^{(-\Delta Ctexp-\Delta Ctcontrol)}$ based on the method described [23,24] and was expressed as fold change in expression using wild-type expression levels as the reference. Gene-specific primers for individual genes were, opgG1_F 5′GCCGATCAGAC GCTAAGTGA and opgG1_R 5′GTTCAAGGCGGGCTTTTACG; opgH2_F 5′ATCTCCCC GGCTCCTATGAA and opgH2_R 5′GGTAGAAAGCGCGAGGAACA; FlhD1_F 5′CTTGCACAGCGTTTGATCGT and FlhD1_R 5′CAGACGCGTTGA AAGCATGA; FlhC1_F 5′GCGC-TCGTCTACAAATGCTG and FlhC1_R 5′TTGCGGAC ACTGCTCAAGAT; FliF1_F 5′AAGAGCACTACAGCCCGAAC and FliF1_R 5′GAGACGCTCAATATCGCCCA; FliA1_F 5′GGTCGACCGATATGACGCTT and FliA1_R 5′TTC-GATGCTATCGCCATGCT; FliC1_F 5′TAC-GCTG-CAA-GTAAAGCCGA and FliC1_R 5′TCGC-GTA-GTCGGAATCTTCG; FliZ2_F 5′TGACG-GTGCAGCAACCTA AA and FliZ2_R 5′GCCATGGCTCATCTCGGTTT; FliT2_F 5′ATCAACCGTTGGCAGC GTAT and FliT2_R 5′GAGTGATTTTTGGCGGGTGG; FlgM1_F 5′GCATTGACCGTAC CTCACCT and FlgM1_R 5′GCG-AATGAGCGAGTCTGCTA; YdiV1_F 5′ATGCTGGC AAATTTT-GGGGC and YdiV1_R 5′CCATAGTCCGCCCTGGAATG; and dnaC1_F 5′TGAG TGCGACGGACAAATGA and dnaC1_R 5′CTCTTCGCTGGTTTCCCGAT.

Results

Swarm and swim motility phenotypes of *opgGH* mutant and medium osmolarity

We examined swarm and swim motility phenotypes of *opgGH* mutant as a function of growth medium osmolarity. For wild type *S. enterica* serovar Typhimurium strain SL1344 cells, a

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bell-shaped curve was observed for swarm motility diameters vs. media osmolarity (**Figure 1**, filled squares). Swarm motility was detected in media >200 mMos Mol^{-1} osmolarity, with optimal swarm motility at 420 mMos Mol^{-1}. Irrespective of medium osmolarity, no swarm motility was exhibited by *opgGH* mutant cells (**Figure 1**, open squares). Under optimal swarm motility conditions (420 mMos Mol^{-1}), the OPG mutant strain generated tiny (8+0.5 mm) swarm radii, compared to much larger (55+3.5 mm) swarm radii of wild-type strain. Unlike swarm motility, swim or individual cell motility (measured as swim diameters in 0.3% semi-solid LB agar media) of wild-type cells was robust over a wide range of osmolarity (**Figure 1** filled circles). Wild-type cells generated swim diameters of 33+2.5 mm to 58+4.1 mm over an osmolarity range of 100-800 mMos Mol^{-1}. Under optimal swarm conditions, the OPG mutant and wild-type strains exhibited indistinguishable swim phenotypes with swim radii of 51+2.5 mm and 54+4.5 mm, respectively. Since no OPG synthesis has ever been reported in growth media with an osmolarity >420 mMos Mol^{-1} [**1**,**3-6**,**25**], the non-swarming phenotype of *opgGH* mutant appeared unexpected and thus, was investigated further.

Requirement of full length *OpgGH* protein for swarm motility

One possible explanation for the perceived requirement of OpgGH proteins for swarming motility could be that proteins remain catalytically silent at high osmolarity may provide mere physical support to maintain membrane stability and integrity.

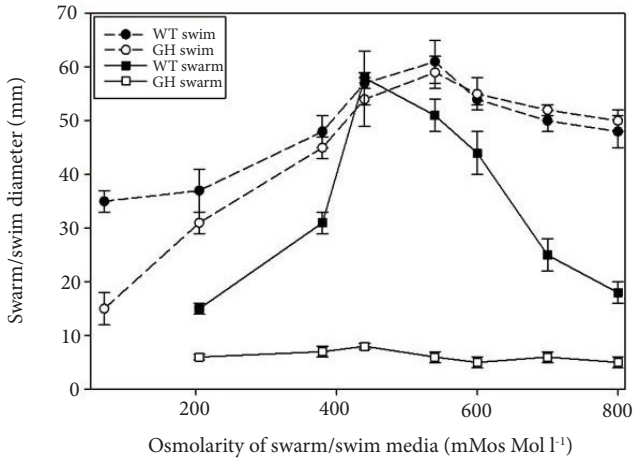

Figure 1. Effect of medium osmolarity (mMos Mol l^{-1}) on swim and swarm motility of S. enterica Serovar Typhimurium wild type and opgGH mutant. Swim motility (●, o) was tested by inoculating 2 µl of overnight grown cells from LB broth in to 0.3% LB-agar plates. Swarm motility (▪, □) was measure by placing 5 µl of overnight grown cells from LB broth on to LB swarm agar plates (0.6% agar, 0.5% glucose in LB medium). Motility diameters were scored after 10 h incubation. Wild-type *Salmonella* SL1344 (●, ▪) and opgGH mutant (o, □). Error bars indicate standard deviation of the mean, and none are shown when the error bars are smaller than the symbol.

Thus it was of interest to examine if the *opgH* gene product, an 847 amino acid protein with estimated molecular weight of 97 kDa, 8 transmembrane segments, and 3 large cytoplasmic regions, might play a role in addition to its catalytic function. Based on its structure, others have proposed that OpgH may form channels to carry OPG to the periplasmic space [**3**,**17**].

Using NEBcutter V2.0 software [**26**], we designed strategies to generate a truncated versions of *opgH* and an in-frame deletion of *opgG*. **Table 1** describes restriction endonucleases used and deleted portions of the *opgG* and *opgH* genes for each of the constructs. pBK16, which carries a full-length wild-type copy of the genes *opgGH,* fully complemented swim motility as well as swarm motility. However, none of the other constructs, such as pMD264 which carries an in-frame deletion of 313 amino acids in the OpgG protein, and intact OpgH or pMD260, which encodes intact OpgG and the first 537 amino acid residues of OpgH, restored swarm or swim motility in low osmolarity media. These observations indicated that full-length gene product with all transmembrane domains is required for a functional swarm phenotype.

Periplasmic glucan biosynthesis protein OpgH is a glucosyltransferase that catalyzes the elongation of beta-1,2 polyglucose chains of glucan, requiring a beta-glucoside as a primer and UDP-glucose as a substrate [**27**]. The central cytoplasmic region of OpgH shares strong structural identity with glucosyl-transferases in which several aspartic acid residues are needed for its catalytic activity [**17**,**28**,**29**]. A ligand-binding site (^{346}Dx^{348}D, accession number cd04191) for OpgH has also been identified in the conserved domain structure database [**30**]. We performed site-directed mutagenesis to change the active site aspartate residues of OpgH at position 346 and 348 to glycine. The plasmid (pAAB13) containing the gene encoding a mutant OpgH protein expressing an altered ligand binding site ^{346}Gx^{348}G on pAAB13 was transfected into OPG-mutant strain GH. Transcription of altered *opgGH* genes was confirmed by performing reverse transcriptase quantitative PCR (data not shown). In spite of successful expression (i.e., transcription), the active-site altered OpgGH failed to support either swim or swarm motility (**Table 1**).

Regulation of flagella synthesis in *opgGH* mutant under swarm growth condition

opgGH mutants are capable of synthesizing active flagella under normal osmolarity liquid growth conditions such as LB broth medium (420 mMos Mol^{-1}) [**20**]. Thus, it was of interest to examine if *opgGH* mutant synthesized flagella under swarm motility conditions. Transmission electronmicrographs of wild-type cells show multiple flagella filaments (10.89+3.9 flagella/cell) as opposed to unflagellated *opgGH* cells (**Figure 2**). This observation is significant because despite the base growth medium remaining the same (i.e., LB broth with osmolarity 420 mMos Mol^{-1}), *opgGH* mutant cells were unable to synthesize flagella, on wet surfaces but had fully functional flagella filaments in liquid media [**20**].

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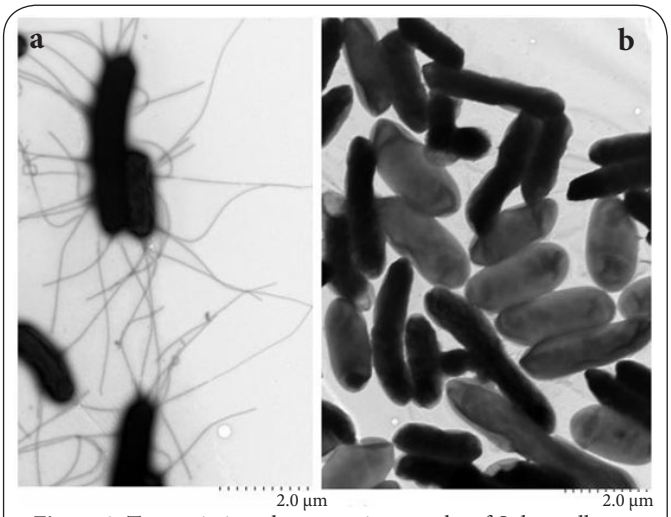

Figure 2. Transmission electron micrographs of *Salmonella enterica* serovar Typhimurium cells from swarm edges. Wild-type SL1344 (**a**) and opgGH mutant (**b**). Bar indicates 2 µm.

In order to gain further insights into the inability of *opgGH* mutant to synthesize flagella we determined transcription levels of several key flagella synthesis regulators [14,31] and compared their levels from liquid culture and swarming growth conditions (**Figure 3**). These regulatory genes are classified based on their transcriptional hierarchy in three stages [31,32]. We examined transcriptional levels of class I regulators (*flhD* and *flhC*), class II regulators *(fliF, fliA, fliT,* and *fliZ*) and class III (*fliC* and *flgM*) in wild type and *opgGH* mutant cells. Corroborating the electron microscopy observation as well as the swarm phenotype, transcriptional levels of flagella synthesis regulons of class II and III were suppressed in *opgGH* mutants when grown on swarm media but not in LB broth cultures. Lack of flagella synthesis in *opgGH* mutants on swarm media does not appear to be due to unavailability of class I master flagella regulatory gene transcripts, namely, *flhD* and *flhC* as their levels were unchanged and comparable to wild-type cells under liquid and swarm condition. Recently an anti-FlhDC factor gene *ydiV*, was suggested to have a role in suppressing motility and flagella regulons [31,32]. Interestingly, levels of *ydiV* gene transcripts were high in *opgGH* mutants compared to wild-type cells under swarm growth conditions ($p<0.001$), but not when compared to liquid LB broth-grown cells (**Figure 3**).

Transcription levels of *opgGH* genes as a function of medium osmolarity

Lastly, we reasoned that although no OPG synthesis has been reported in media with osmolarity >420 mMos Mol^{-1} [5,6], there might be a low level of OPG synthesis below the detection limits. To this end, we monitored the transcript levels of *opgG* and *opgH* genes at various osmolarity (**Figure 4**). In order to maximize OPG synthesis, researchers have used nutrient sufficient but hypoosmotic conditions (i.e., LB with-out salts, ~100 mMos Mol^{-1}) to grow *E. coli, S. flexneri* and *Salmonella* sp. [5,6,28]. Normalizing the *opgGH* transcript levels to LB-no salts growth conditions, we compared transcript levels from wild-type cells grown in LB containing up to 0.8 M NaCl. Quantitative reverse transcriptase –PCR of *opgG* and

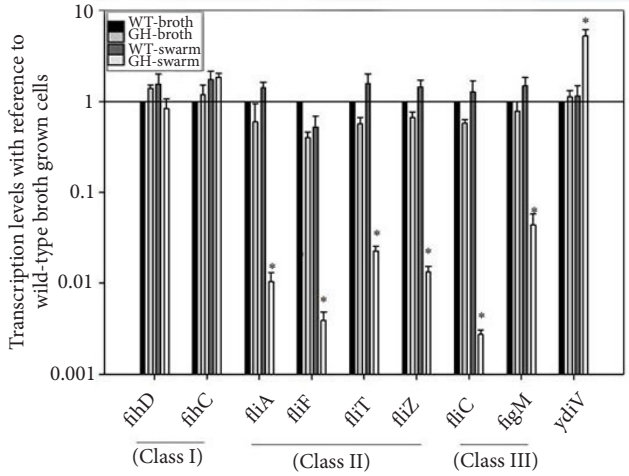

Figure 3. Transcription levels of flagella regulatory genes in liquid and swarm growth conditions. Expression levels of individual genes were referenced to the expression values from wild-type cells under liquid growth condition (black bars). Wild-type swarming cells (dark grey), opgGH liquid culture cells (grey bars) and swarm cells (light grey bars). For individual genes, expression values appear in the order of wild-type liquid culture cells (reference), opgGH liquid culture cells, wild-type swarm cells and opgGH swarm cells. *indicates values with statistically significant difference compared to wild-type liquid culture transcripts ($p<0.001$).

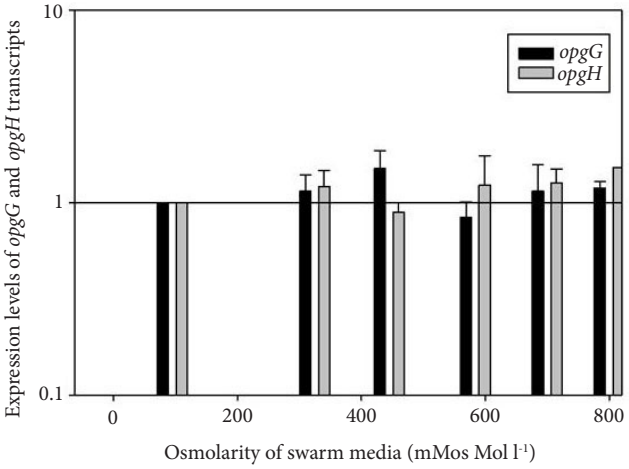

Figure 4. Effect of swarm media osmolarity (mMos Mol l^{-1}) on opgG and opgH transcripts levels in S. enterica serovar Typhimurium SL1344 wild-type cells. Expression levels from cells grown in LB medium without salts (95 mMos Mol^{-1}) was used as reference.

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opgH transcripts indicated that cells continue to synthesize *opgGH* RNA in spite of high osmolarity of the surrounding growth media (**Figure 4**).

Discussion and conclusion

Osmoregulated periplasmic glucans, OPG, are abundantly synthesized in low osmolarity media (ca. 100 mMos Mol^{-1}) and their synthesis progressively diminishes at higher osmolarity growth conditions [**3**,**5**,**6**,**28**,**33**]. In spite of high levels of *opgGH* RNA transcripts (**Figure 4**), no OPG are detected in cells grown on regular LB broth media [**5**,**6**]. In concurrence with these observations, pleiotropic effects observed in *opg* mutants such as compromised swim motility and growth rate in low osmolarity media are compensated by adjusting the osmolarity of the external growth media [**12**,**34**]. Since swarm motility is detected in media with osmolarity >400 mMos Mol^{-1} [**14**,**16**], lack of swarm motility by *opgGH* mutant was unanticipated and was the subject of investigation of this study.

Data from experiments involving truncated and catalytically silent OpgGH constructs indicated the requirement for a full-length, functionally active product for swarm motility (**Table 1**). No evidence was found in support of the possibility that for swarm motility, the sole role of OpgGH proteins was to lend physical support for membrane integrity and structure while remaining catalytically silent. On the contrary, high transcription levels of *opgGH* under swarm growth conditions (**Figure 4**) suggest that OPG may continue to be synthesized (below current detection limits) to support functions such as swarming motility. Earlier we reported that under low osmolarity conditions only 17% of the *opgGH* mutant cells synthesized flagella compared to 100% flagellated cells of wild-type [**20**]. Among the flagellated cells, *opgGH* mutant harbored 1.18+0.4 flagella per cell (wild-type cells synthesized 3.18+1.52 flagella per cell). On the contrary, when grown in nutrient sufficient condition such as in LB broth, 100% of the mutant cells harbored flagella and there was no significant difference in number of flagella per cell compared to wild-type cells (4.46+2.1 and 4.9+2.16 for mutant and wild-type, respectively) [**20**]. Expression of flagella regulators was unchanged in *opgGH* mutant cells grown in liquid LB broth cultures (**Figure 3**) and supports our previous observation that individual swim motility and number of flagella per cell remain unaffected when mutant cells were grown in LB broth media. On the other hand, under swarming growth condition, lack of OPG synthesis resulted in cells with significant suppression of class II and III flagella regulatory genes (**Figure 3**).

Swarming motility is an energy-intensive process and requires integration of many environmental cues triggering physiological signaling networks [**15**,**16**]. It was observed that the *opgGH* mutant had increased levels of gene transcripts of *ydiV* (**Figure 3**) encoding an anti-FlhDC factor [**31**]. In *Salmonella* high levels of YdiV were considered to be a response to poor nutrient conditions and the class-II regulatory gene, *fliZ*, was considered to be a repressor of *ydiV* [**32**]. We observed low levels of *fliZ* with concomitant induced levels of *ydiV* (**Figure 3**). This observation is in agreement with the anti-FlhDC role assigned for YdiV and further corroborated by the fact that *opgGH* mutant cells are unflagellated on wet surfaces (**Figure 2**). However, high levels of *ydiV* transcripts under swarm growth conditions clearly suggest that involvement of YdiV may not be limited to poor nutrient conditions. YdiV has a weak EAL domain and it is likely that the protein is involved in degradation of the secondary messenger molecule cyclic di-GMP [**35**]. It is postulated that the lack of OPG results in cells incapable of coordinating surrounding environmental stimuli due to perturbed secondary messenger pathways. Data presented here suggest that OPG have roles beyond periplasmic stability in low osmolarity environments and defining it will require further investigations such as whole transcriptome (RNASeq) analyses under swarming condition.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	MSD	PK	CM	ADS	AAB
Research concept and design	✓	--	--	--	✓
Collection and/or assembly of data	✓	✓	✓	--	✓
Data analysis and interpretation	✓	--	--	✓	✓
Writing the article	--	--	--	✓	✓
Critical revision of the article	--	--	--	✓	✓
Final approval of article	--	--	--	✓	✓
Statistical analysis	--	--	--	✓	✓

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