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Sébastien Bontemps-Gallo, Jean-Marie Lacroix

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New Insights into the Biological Role of the Osmoregulated Periplasmic Glucans in Pathogenic and Symbiotic Bacteria

Sébastien Bontemps-Gallo[1] and **Jean-Marie Lacroix**[2]

[1]Laboratory of Zoonotic Pathogens, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 903 South 4th Street, Hamilton, MT, 59840, USA. Phone: +1 (406) 363-9259. sebastien.bontemps-gallo@nih.gov

[2]Structural and Functional Glycobiology Unit, UMR CNRS-Lille1 8576, University of Lille, 59655 Villeneuve d'Ascq cedex, France. Phone: +33 3 20 43 65 92. jean-marie.lacroix@univ-lille1.fr

Summary

This review emphasizes the biological roles of the osmoregulated periplasmic glucans (OPGs). OPGs occur in almost all α, β and γ Proteobacteria. This polymer of glucose is required for full virulence. The roles of the OPGs are complex and vary depending on the species. Here, we outline the four major roles of the OPGs through four different pathogenic and one symbiotic bacterial models (*Dickeya dadantii, Salmonella enterica, Pseudomonas aeruginosa, Brucella abortus* and *Sinorhizobium meliloti*). When periplasmic, the OPGs are a part of the signal transduction pathway and indirectly regulate genes involved in virulence. The OPGs can also be secreted. When outside of the cell, they interact directly with antibiotics to protect the bacterial cell or interact with the host cell to facilitate the invasion process. When OPGs are not found, as in the ε Proteobacteria, OPG-like oligosaccharides are present. Their presence strengthens the evidence that OPGs play an important role in virulence.

Introduction

First discovered in 1942 in the analysis of a culture supernatant of *Agrobacterium tumefaciens* (McIntire *et al.*, 1942), the osmoregulated periplasmic glucans (OPGs, formerly called membrane-derived oligosaccharides, MDOs) are described as an exopolysaccharide subclass. In 1973, Kennedy's lab rediscovered these glucans in a study on the membrane phospholipid turnover in *Escherichia coli* (Van Golde *et al.*, 1973). During this study, Van Golde and collaborators demonstrated that the free phosphoglycerol from the membrane phosphatidylglycerol turnover is transferred onto free soluble oligosaccharide in the periplasm (Van Golde *et al.*, 1973) and called this oligosaccharide membrane-derived oligosaccharide (MDO).

Features of the osmoregulated periplasmic glucans

Two common features clearly define the osmoregulated periplasmic glucans: 1) the sole sugar is a D-glucose and 2) the main backbone is based on β-linkage. Except in *Brucella abortus*, the concentration of the OPGs increase when the osmolarity decreases (Bohin and Lacroix, 2006). Because not all the MDOs display substitution from the membrane phospholipids, they were renamed, in 2000, osmoregulated periplasmic glucans (Bohin,

2000). The OPGs occur in almost all the α, β and γ Proteobacteria and display an interesting structural diversity (Figure 1).

Structure of the osmoregulated periplasmic glucans

The OPGs were divided into four families based on their structure (Bohin, 2000). The most studied family is family I, which is synthesized by a variety of species including *Escherichia coli*, *Salmonella enterica* and *Dickeya dadantii*. The OPG family I is a linear glucan containing 5–12 glucose units joined by β-1,2 linkages and branched by β-1,6 linkages. Found in the *Agrobacterium*, *Sinorhizobium* and *Brucella species*, the OPGs family II is a cyclic glucan containing 17–25 glucose units joined by β-1,2 linkages. Both last two families (families III and IV) – found in *bradyrhizobium* - are also cyclic, containing 10–28 glucose units joined by various kinds of β and α linkage. The glucose backbone of OPGs can be substituted by various kind of molecules depending on the species, *O*-succinyl residues being the most widely distributed (see Bohin, 2000 for a review on structural diversity of OPG).

The synthesis of the glucose backbone requires either the *opgGH* operon (families I and IV) (see Bontemps-Gallo *et al.*, 2013a for a biosynthesis working model) or *ndvAB/chvAB/cgs* operon (families II and III). The inactivation of the operon provokes a total loss of osmoregulated periplasmic glucans (Bohin, 2000; Bohin & Lacroix, 2006).

Pleiotropic phenotype of the OPG mutant strain

OPG mutant strains devoid of OPG display a pleiotropic phenotype. The phenotype depends on the bacterial species (Table 1). In all the phenotypes, one is consistent: the loss of virulence for both phyto- and zoo-pathogenic bacteria.

Here, we outline the four major biological roles of the osmoregulated periplasmic glucans through 5 main organism models.

In *Dickeya dadantii* and *Salmonella enterica*, the osmoregulated periplasmic glucans are required for virulence through the perception of the environment

The first studies on the OPGs biological role was done on the non-pathogenic *E. coli* K12, which shows only a few phenotypes (Bontemps-Gallo *et al.*, 2013a). The inactivation of either *opgG* or *opgH* gene provokes a decrease of the motility and an increase of the mucoidy in *E. coli* (Bontemps-Gallo *et al.*, 2013a). New study models were required for a better understanding of the OPGs biological role in virulence. Two models were developed: *Dickeya dadantii* by our lab and *Salmonella enterica* by Bhagwat's lab.

Dickeya dadantii (formerly *Erwinia chrysanthemi*) is a necrotophic phytopathogenic Enterobacterium. It is a causative agent of soft-rot diseases affecting a wide range of plant species. *Dickeya spp*. are responsible for a quarter of the losses of potato crops in Europe (Toth *et al.*, 2011). The infection of *D. dadantii* is based on the secretion of a set of plant cell wall degrading enzymes (PCWDEs) (Reverchon and Nasser, 2013).

Salmonella enterica is an intracellular human pathogenic strain. *S. enterica* pathogenesis is based on the capacity to invade a non-phagocytic cell and replicate inside a vacuole (Steele-Mortimer, 2008).

In *D. dadantii* and *S. enterica*, the concentration of the OPGs increases when the osmolarity decreases (Cogez *et al.*, 2001; Bhagwat *et al.*, 2009). Inactivation of the *opgGH* operon in both species provokes a total loss of virulence (Page *et al.*, 2001; Bhagwat *et al.*, 2009; Liu *et al.*, 2009). In 2007, a comparative proteomic study between the wild-type and the OPG defective strain in *D. dadantii* showed a general stress response manifested as general metabolism perturbation (Bouchart *et al.*, 2007). A similar proteomic study in *Salmonella* completed five years later (Cooper *et al.*, 2012), confirmed a strong perturbation in the fitness of the bacteria. Furthermore, the phenotypes of both species (Table 1) and both proteomic studies suggest an impairment in their ability to perceive changes in their environment.

In *D. dadantii*, a nitrosoguanidine-induced mutagenesis was performed on the *opg* strain and restoration of motility was used as a screen. A mutation in the Rcs system was found to restore the motility and the virulence only in potato tubers (Bouchart *et al.*, 2010). The Rcs phosphorelay is a complex signaling pathway. When the Rcs system is activated, it represses genes involved in virulence, motility, mucoidy and the expression of the PCWDEs through the Rsm system (Majdalani & Gottesman, 2005; Clarke, 2010, Wu *et al.*, 2014). Changing the periplasmic concentration of the OPGs *in vitro* directly changed the level of activation of the Rcs system (Bontemps-Gallo *et al.*, 2013; Madec *et al.*, 2014). The OPGs are directly involved in the perception of the environment through the Rcs phosphorelay (Figure 2A). Furthermore, throughout the infectious process, the concentration of the OPGs increases, thereby repressing the Rcs phosphorelay (Bontemps-Gallo *et al.*, 2013; Madec *et al.*, 2014). In turn, this repression allows the expression of the motility and the PCWDEs genes. In *opg* strains, the decrease of the motility is the result of the repression of the *flhDC* master operon by the Rcs system, which control the expression of the flagellum structural gene (Bouchart *et al.*, 2010; Bontemps-Gallo *et al.*, 2013; Madec *et al.*, 2014). At the same time in the *opg* strain, the activation of the Rcs system provokes the increase of the mucoidy to protect the cell against high osmolarity (*i.e.* desiccation) and ROS stresses (Bontemps-Gallo *et al.*, 2014). Defects in strains devoid of OPGs are not restricted to the Rcs control. In *D. dadantii*, a study on the relationship between the OPGs and the major virulence factor demonstrated that *opg* strains displaying an inactivated Rcs system are rapidly killed by plant defense and are unable to overcome the repression by PecS, the main repressor of virulence in *D. dadantii* (Bontemps-Gallo *et al.*, 2014).

Furthermore, recently, an additional feature of the pleiotropic phenotype was discovered by Hill and collaborators in *E. coli*. The OpgH glucosyltransferase (catalyzing the linear β-1,2 glucose linkages of OPGs) interacts with the FtsZ protein, the major component of cell division ring (Hill *et al.*, 2013). By sequestering FtsZ, OpgH directly modulates the division and the size of the cell depending on the medium nutrient availability.

The alteration of the perception of the environment associated with the global disorder of the internal control system provoked by the loss of the OPGs led us to use the term of bacterial autism for the *opg* strain (Bontemps-Gallo *et al.*, 2014).

Here, the OPGs play two main roles: a structural role and a role in environmental perception. The OPGs help to maintain the osmotic pressure in the periplasm required for the cell (Bohin & Lacroix, 2006; Sochacki *et al.*, 2011) and at the same time regulate – at least for *D. dadantii* – the virulence through the Rcs phosphorelay and the PecS repressor.

In *Brucella abortus*, the secretion of the osmoregulated periplasmic glucans is required for an early step in host cell invasion

Brucella abortus, causative agent of brucellosis, is an intracellular pathogen which causes premature abortion of cattle and human fetuses. To achieve the invasion of its host, *B. abortus* has to establish itself inside of the host cells before it can replicate (Gorvel and Moreno, 2002). After interaction with the host cell lipid rafts, *B. abortus* is internalized in the vacuole. *B. abortus* then remodels the vacuole to transiently allow a fusion with the lysosome. These interactions between the vacuole and the lysosome provoke a decrease in pH, which is required for the activation of the acid-dependent genes of *B. abortus* and in particular the type 4 secretion system (T4SS). The T4SS secretes into the cytoplasm of the host cell a set of effectors allowing the interaction of the vacuole with the endoplasmic reticulum (ER). The vacuole displays some ER markers and after that, *B. abortus* can initiate the replication process inside of the vacuole (Gomez *et al.*, 2013).

In *B. abortus*, the OPGs or β-1,2 cyclic glucans (CβG) are secreted into the vacuole and are not regulated by the osmolarity (Roset *et al.*, 2004, Arellano-Reynoso *et al.*, 2005). The OPGs are involved only in the early step of the invasion (Figure 2B). The OPGs interact with the lipid rafts of the cell host to allow the maturation of the vacuole, and are required for the fusion with the lysosome, but they are not required after this step (Arellano-Reynoso *et al.*, 2005).

Interestingly, Gorvel's lab also described that the OPGs of *B. abortus* are non-immunogenic but can activate the dendritic cells and could be used as a new class of adjuvants (Martirosyan *et al.*, 2012).

In *Pseudomonas aeruginosa*, the osmoregulated periplasmic glucans enhance biofilm formation and confer a higher antibiotic resistance

Pseudomonas aeruginosa, an opportunistic pathogen, is mostly studied in connection with its infection of immunodeficient or cystic fibrosis (CF) patients. The persistence of the infection is dependent upon its biofilm lifestyle. *P. aeruginosa* has the distinctive feature of possessing both *opgGH* and *ndv* operons (Mah *et al.*, 2003; Lequette *et al.*, 2007). Both linear and cyclic glucans are synthesized in *P. aeruginosa*: linear OPGs from the *opgGH* operon and β-1,3 cyclic glucans from *ndv* genes (Mah *et al.*, 2003; Lequette *et al.*, 2007; Sadovskaya *et al.*, 2010). The loss of the linear OPGs reduces biofilm development (Lequette *et al.*, 2007). However, the *ndv* operon produces cyclic glucans, which directly

interact with the aminoglycoside antibiotics inside the biofilm (Figure 1C)(Mah *et al.*, 2003; Sadovskaya *et al*., 2010). By sequestering the antibiotic molecules in the biofilm, the bacteria prevent the antibacterial molecules from arriving at their cellular targets. Furthermore, the *ndvB* gene is required for the expression of the ethanol oxidation genes needed for some types of antibiotic resistance (Beaudoin *et al*., 2012). Directly or indirectly, the cyclic OPGs are involved in antibiotic resistance while the linear OPGs are involved but not essential for biofilm formation.

In *Sinorhizobium meliloti* and *Bradyrhizobium japonicum*, the loss of the osmoregulated periplasmic glucans distorts nodule development

Both *Sinorhizobium meliloti* and *Bradyrhizobium japonicum* live in a symbiotic relationship with legumes inside of root nodules. Only bacteria are able to fix atmospheric nitrogen. However, the nitrogenase required for this process is sensitive to oxygen. The nodule is developed by the plant in response to nod factors produced by the bacteria and offers a microaerobic environment for the bacteria (Gage, 2004).

Geremia and colleagues demonstrated that in *S. meliloti* the loss of the osmoregulated periplasmic glucans prevented them from invading the cortical cells, though the plants were still able to elicit nodule formation (Geremia *et al.*, 1987). About ten years later, Dunlap and colleagues showed that *Bradyrhizobium japonicum* defective OPG mutant strain expressed nodule genes at lower levels than normally required for the colonization of plant roots by the wild type strain. Furthermore, late nodule genes are also delayed in expression (Dunlap *et al.*, 1996). Bacteria devoid of OPGs are able to induce nodule formation but when the plant defenses increased, bacterial colonization of the nodule stops and leads to empty nodules (Dunlap *et al*., 1996). Outside the host, loss of OPGs leads to a loss of osmoadaptation/ osmoremediation by the addition of various ionic or non ionic molecules (Dylan *et al.*, 1990b).

These studies show that the osmoregulated periplasmic glucans are involved in nodule development, in osmoadaptation, and seem to be involved in the response to plant defenses.

OPG-like: free oligosaccharide (fOS) of *Campylobacter jejuni*

OPGs were described in α, β and γ Proteobacteria. Recently, Szymanski's lab published two articles on the free oligosaccharide (fOS), where Nothaft and colleagues demonstrated that the fOS plays a similar role in *Campylobacter jejuni* to that of the OPGs in *D. dadantii*. These oligosaccharides are released by hydrolysis of the intermediates of N-glycosylation pathways (Nothaft *et al*., 2009). Synthesized on the bactoprenol, an important amount of these oligosaccharides end up free in the periplasmic space instead being transferred to the glycoproteins. The concentration of the fOS increases when the osmolarity decreases (Nothaft *et al*., 2009; Nothaft *et al*., 2010). Furthermore, the inactivation of the gene involved in the biosynthesis of the fOS provokes a loss of virulence (Nothaft *et al.*, 2010) similar to the inactivation of the OPG biosynthesizing gene. The presence of this OPG-like oligosaccharide strongly suggests that the presence of the periplasmic glucans is essential for almost all the Proteobacteria.

Conclusion

The goal of this review is to offer an overview of the different biological roles of the osmoregulated periplasmic glucans in pathogenic and symbiotic bacteria.

OPGs are secreted by a few bacterial species. Secreted OPGs can directly bind the aminoglycosides to prevent the antibiotics from reaching the cell target in *P. aeruginosa* (Mah *et al.*, 2003; Sadovskaya *et al.*, 2010). They can also directly interact with the phagosome to allow fusion with the lysosome, a step required for successful virulence of *B. abortus* (Arellano-Reynoso *et al.*, 2005).

Even if secreted, at least part of OPGs are periplasmic, and their presence is not restricted to pathogenic or symbiotic bacteria. In *E. coli* K12, the EnvZ-OmpR system seems to be affected by the loss of the OPG (Fiedler & Rotering, 1988). Motility and mucoidy are also affected in *E. coli* K12, as observed in *D. dadantii* and *S. enterica*, strongly suggesting that the Rcs system depends on OPGs in this non pathogenic bacterial species. Taken together, these two examples demonstrate that the OPGs can be a part of signal transduction, regardless of whether the bacteria is pathogenic or saprophytic, either by transmitting the signal from the outer membrane to the inner membrane or by affecting the membrane fluidity (Clarke and Voigt, 2011). Interestingly, the Rcs System is found only in the enterobacteria but several common phenotypes are also described in the Rhizobiaceae (α-Proteobacteria) which are devoid of OPGs, such as an overexpression of exopolysaccharides or loss of motility (Breedveld *et al.*, 1994). Furthermore, little is known about the implication of the OPGs as regards environmental sensing for these bacteria. Are there two-component systems affected by the OPGs concentration outside of the Enterobacteriaceae ?

The second major role of periplasmic OPGs is in the structure of the cell envelope, as described in *E. coli* as well as in *D. dadantii* or *S. enterica* (Bohin & Lacroix, 2006). The recent study from Hill and collaborators shows a direct involvement of the OpgH protein in cell division (Hill *et al.*, 2013). What is exactly the role of this interaction? OpgH could play a similar role in other Enterobacteria but does it also happen in the other bacterial families? *e.g.* do *ndvB* mutants show a similar phenotype? More investigations are required to understand the role of this interaction with cell division.

Finally, the OPGs occur only in the α, β and γ Proteobacteria. The OPG-like oligosaccharides found in the ε-proteobacteria suggest that the presence of periplasmic glucans is essential but no OPG or OPG-like oligosaccharides was found in the δ or in the ζ Proteobacteria. This prompts us to ask about the origin of the OPG genes. The α, β and γ Proteobacteria have emerged later than the δ, ε and ζ Proteobacteria (Emerson *et al.*, 2007). One can hypothesize that this acquisition was an evolutionary advantage, which allowed these genes to spread in almost all the α, β and γ Proteobacteria. The origins of the acquisition as well as the advantage conferred on the OPG biosynthesis genes acquisition are still questions.

Lastly, the last 15 years offer a clear new insight in the understanding of the biological roles of the OPGs in pathogenic and symbiotic bacteria. However, this only represents the tip of the iceberg and a snapshot of what we imagine to be one important molecule for general cell

physiology. Probably as a consequence of the grants system (*i.e.* more money spent on disease), little is known of the role of the OPGs in the nonpathogenic bacteria. Studies on nonpathogenic bacteria and specially outside of the enterobacteria have to be done to shed light on the biological role of the OPGs.

Outlook: OPG: a target for new antimicrobial molecules?

The OPGs occur in many of the zoo- and phyto- pathogenic bacteria. Because the loss of the OPGs provokes the loss of virulence, inhibiting the biosynthesis of the OPGs should help the host immune system to eliminate the pathogen. Stopping OPGs synthesis in order to fight an infection will require a better understanding of the OPGs biosynthesis, in particular how OpgH/OpgG or NdvA/NdvB interact and transfer the glucose from the UDP-glucose into linear branched or cyclic OPGs.

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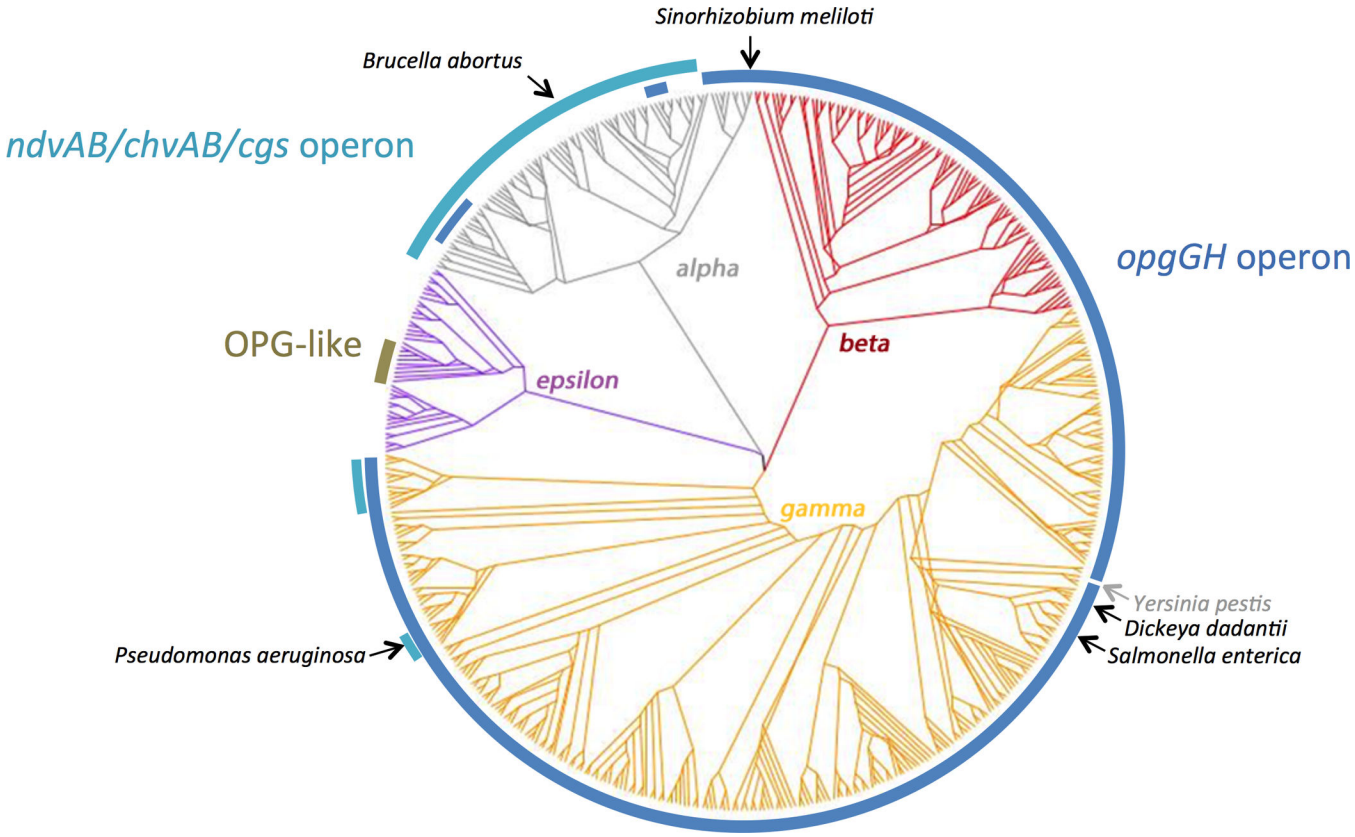

Figure 1. Distribution of linear, cyclic OPG and OPG-like oligosaccharide

Presence of genes encoding linear, cyclic OPG or OPG-like oligosaccharide are mapped onto a phylogenetic tree of species with completely sequenced genomes from the MicrobesOnline database (http://www.microbesonline.org - Dehal *et al.*, 2010). Different classes of the Phylum of the Proteobacteria are colored as follows: grey, α-proteobacteria; red, β-proteobacteria; violet, ε-proteobacteria; yellow, γ-proteobacteria. The ring surrounding the tree shows the presence of linear OPG (dark blue, presence of *opgGH* operon), cyclic OPG (light blue, presence of *ndvAB/chvAB/cgs* operon), OPG-like oligosaccharide (maroon, only in the genus of *Campylobacter*). The black arrows indicate the four pathogenic and the symbiotic bacterial models described in this review. The grey arrow indicates *Yersinia pestis*, which lost the *opgGH* operon.

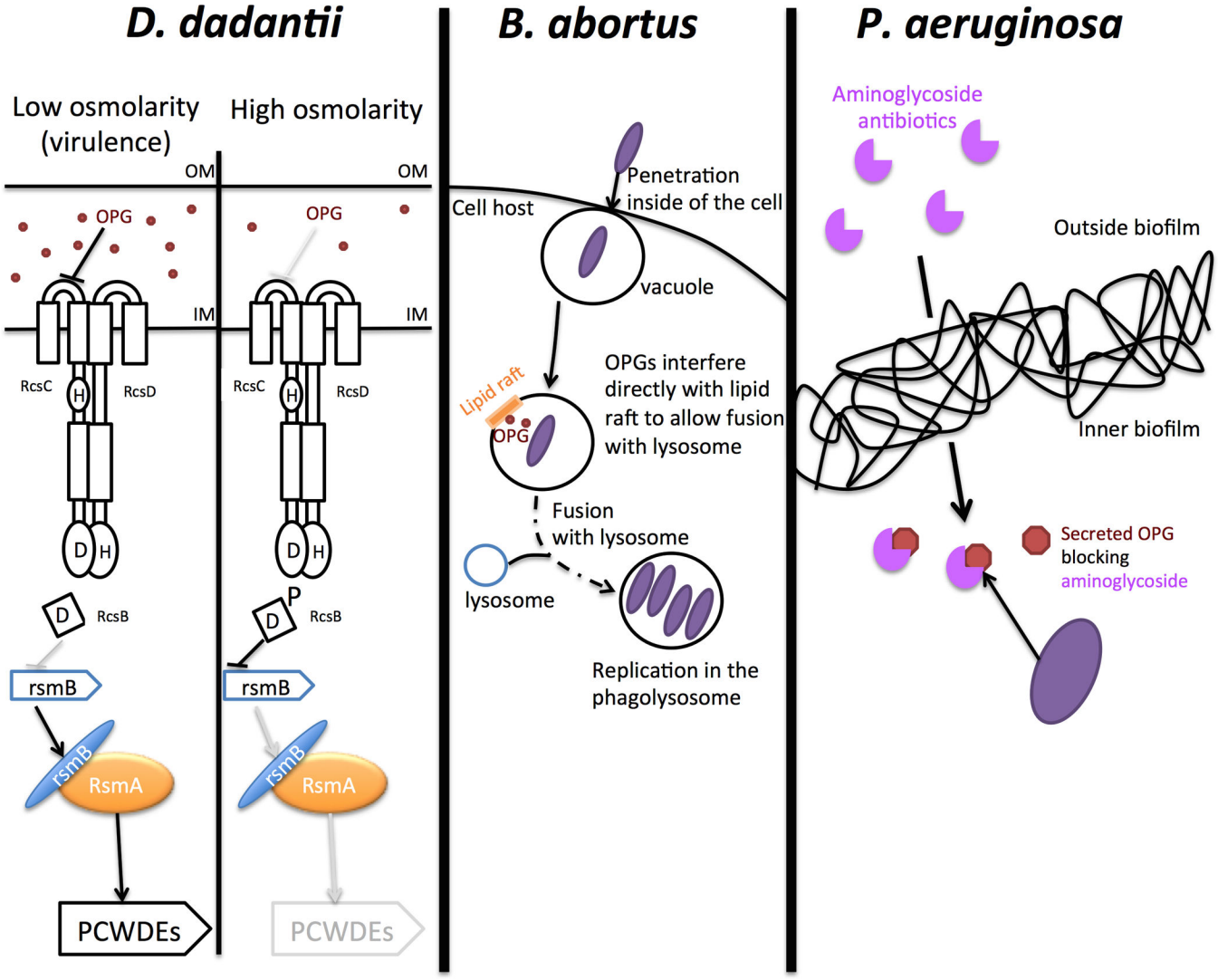

Figure 2. Working Model of the OPG role in *D. dadantii* (A), *B. abortus* (B) and *P. aeruginosa* (C)

(A) In *D. dadantii*, the high concentration of the OPG represses the RcsCDB system at low osmolarity which avoids the repression of *rsmB* by RcsB phosphorylated stage. *rsmB* small RNA encoding by *rsmB* can form a complex with the RsmA protein and enhance the expression of the PCWDEs.

(B) After the penetration of the host cell inside of a vacuole, *B. abortus* needs to merge with the lysosome to be able to replicate. The fusion of the vacuole with the lysosome is dependent upon the interaction between the cyclic OPG and the vacuole's lipid raft.

(C) In *P. aeruginosa*, the cyclic OPGs are secreted within the biofilm and are able to bind the aminoglycoside antibiotics. By sequestering the antibiotics, the cyclic OPGs prevent the aminoglycoside from reaching the cell target.

Table 1
Phenotype described by the loss of the OPGs

In grey, the non-pathogenic species; in green, the phytopathogenic species; in pink, the zoopathogenic species; in violet, the symbiotic species.

Phenotype	Species	Observation	References
Mucosity	*Escherichia coli*	Overproduction of the exopolysaccharides	Ebel *et al.*, 1997
	Dickeya dadantii		Page *et al.*, 2001
	Rhizobiaceae		Breedveld *et al.*, 1994
Motility	*Escherichia coli*	Loss of motility due to a deficit in flagellum (Repression of the *flhDC* master operon by the Rcs system)	Fiedler and Rotering, 1998
	Dickeya dadantii		Page *et al.*, 2001
	Salmonella enterica sv Typhimurium		Bhagwat *et al.*, 2009
Cell division	*Escherichia coli*	Sequestration of FtsZ	Hill *et al.*, 2013
Growth in hypoosmotic medium	*Sinorhizobium meliloti*	Slow growth in low osmolarity	Breedveld *et al.*, 1994
	Agrobacterium tumefaciens		Miller *et al.*, 1986
	Salmonella enterica sv Typhimurium		Bhagwat *et al.*, 2009
	Shigella flexneri		Liu *et al.*, 2010
Sensitivity	*Sinorhizobium meliloti*	Antibiotics	Dylan *et al.*, 1990
	Pseudomonas aeruginosa		Mah *et al.*, 2003
	Escherichia coli	SDS	Rajapogal *et al.*, 2003
	Dickeya dadantii	Bile salts	Page *et al.*, 2001
Biofilm	*Dickeya dadantii*	Loss of the capacity to form a biofilm	F. Bouchart, 2006
	Pseudomonas aeruginosa	Slow formation of the biofilm	Lequette *et al.*, 2007
Virulence	*Brucella abortus*	Perturbation in the invasion of the host cell	Briones *et al.*, 2001 Roset *et al.*, 2004 Arrellano-Reynosa *et al.*, 2005
	Yersinia enterocolitica	OpgH is required in the early stage of the infection	Young and Miller, 1997
	Pseudomonas aeruginosa	Loss of virulence in mice, *Caenorhabditis elegans* and *Arabidopsis thaliana*	Mahajan-Miklos *et al.*, 1999
	Salmonella enterica sv Typhimurium	Loss of virulence in mice	Bhagwat *et al.*, 2009
	Shigella flexneri	Loss of the capacity to infected macrophages and HeLa cells	Liu *et al.*, 2010
	Pseudomonas syringae	Loss of virulence	Loubens *et al.*, 1992, 1993
	Dickeya dadantii	Loss of virulence in chicory leaves, on potato tubers, on carrot	Page *et al.*, 2001 Unpublished data
	Agrobacterium tumefaciens	Loss of virulence	Puvanesarajah *et al.*, 1985
	Xanthomonas campestris	Loss of virulence on tomato leave.	Minsavage *et al.*, 2004
Symbiosis	*Sinorhizobium meliloti*	Perturbation in the root nodule development	Geremia *et al.*, 1987
	Bradyrhizobium japonicum		Dunlap *et al.*, 1996