REUNIÓN CONJUNTA DE SOCIEDADES DE BIOCIENCIAS

Abstract ID: 1472

Bordetella bronchiseptica LapD interacts with two different diguanylate cyclases

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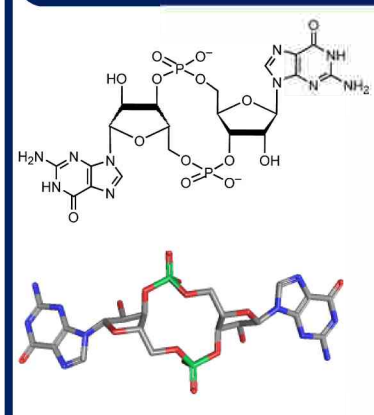
1. What is c-di-GMP?

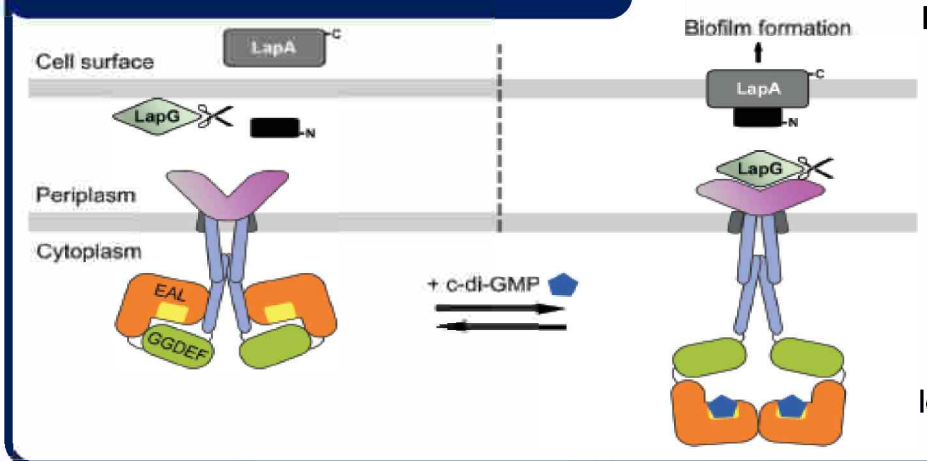


Cyclic-di-GMP (cdG) is a widely distributed second messenger in the bacterial world. It is produced by diguanylate cyclases (DGCs) and can direct many cellular processes like motility and biofilm formation among others.

2. Bordetella bronchiseptica (*Bb*)

This is a Gram-negative pathogen that causes airway tract infections in a variety of mammals including humans. It is closely related to the whooping cough-causing agent *B. pertussis*.

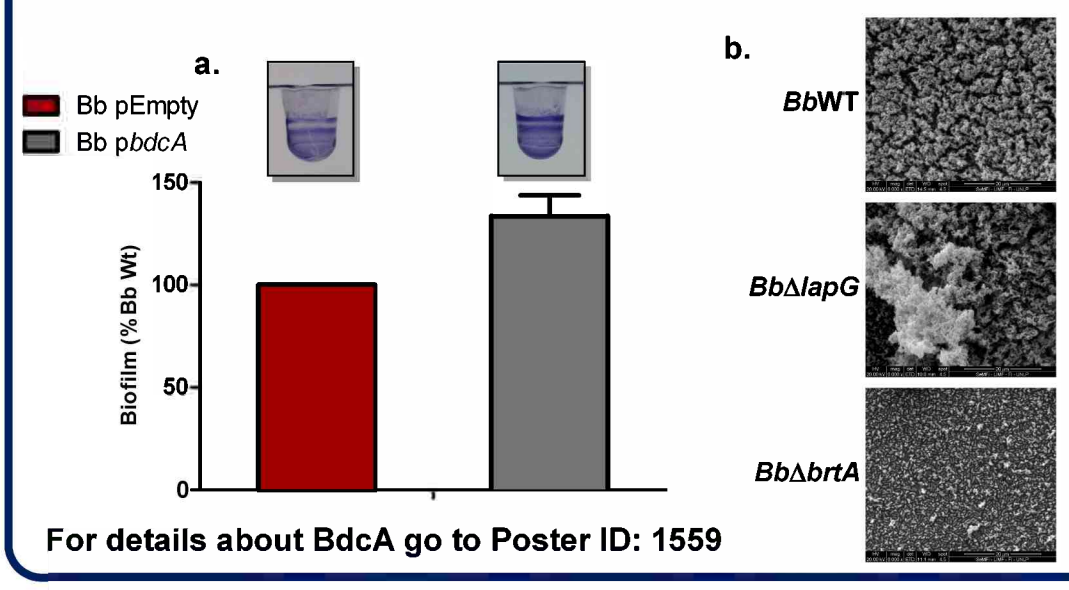
3. Lap proteins



Lap proteins regulate biofilm formation in in a variety of species. c-di-GMP sensor LapD controls LapA localization through periplasmic protease LapG in *Ps. Flourescens*[1]. In *Bb,* LapG and LapD homologs control the localization of the adhesin BrtA[2].

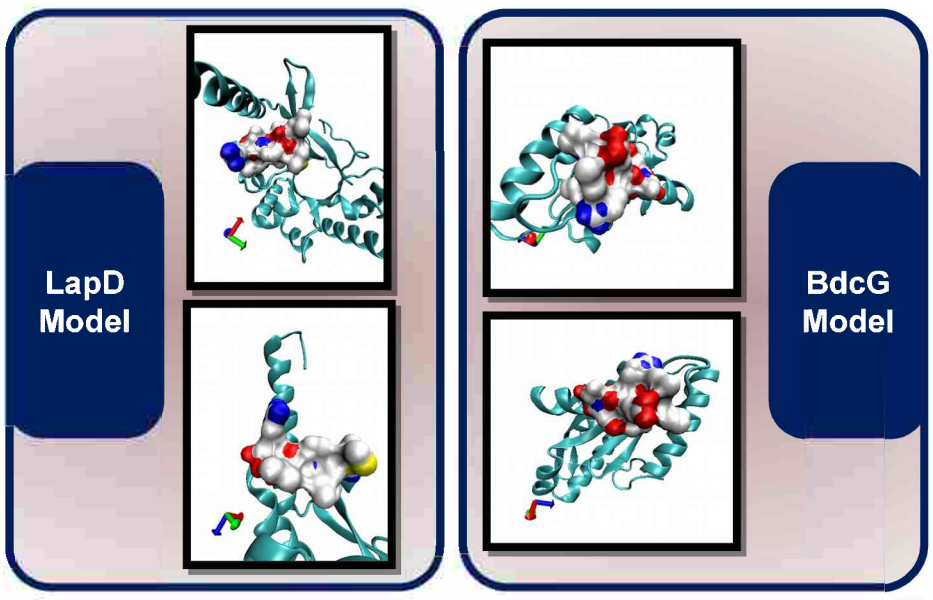
4. Previous results

cdG regulates biofilm formation probably through Lap proteins. a. c-di-GMP regulates biofilm formation in *Bb.* BdcA overproduction enhances biofilm formation[3]. Results were normalized against Bb pEmpty. **b.** Scanning Electron Microscopy (SEM) show the involvement of LapG and BrtA in *Bb* biofilm formation[1].



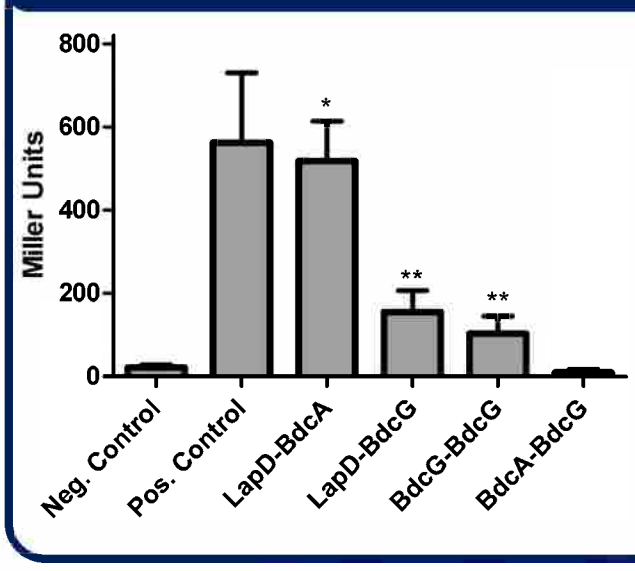
For details about BdcA go to Poster ID: 1559

5. Predictive Model of LapD-DGC interactions



Bioinformatic approach. Using the protein folding recognition and modeling server, Phyre 2[4] we have looked into the interaction regions of LapD and every membrane *Bb* DGC. Based on Dahlstrom and co-workers research[5] we have predicted that BdcG might physically interact with LapD.

6. BdcA and BdcG physically interact with LapD



Bacterial Two Hybrid experiments (BATCH). β-galactosidase activity expressed in Miller Units reflects the interaction between assessed proteins. * or **, indicate a significant difference between the assessed couple of proteins and the negative control, $p<0.01$ or $p<0.05$ respectively.

7. BdcG is not an active DGC under tested experimental conditions

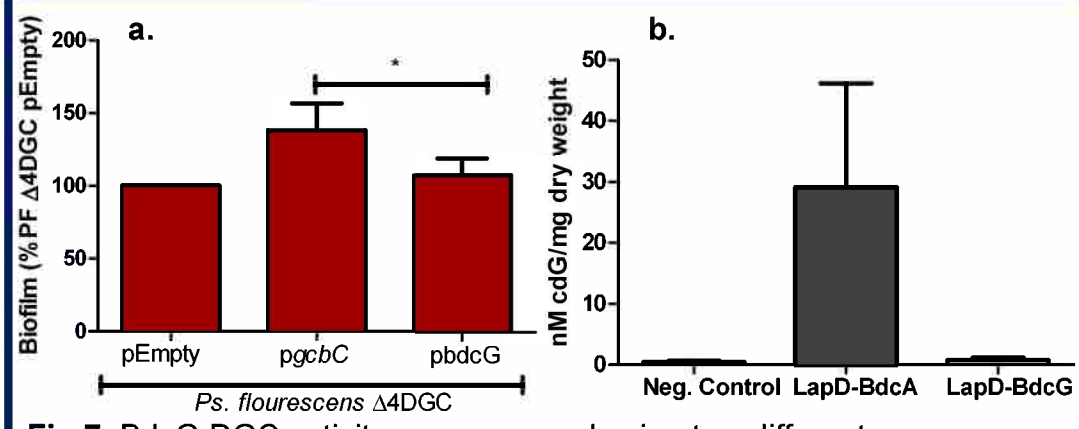
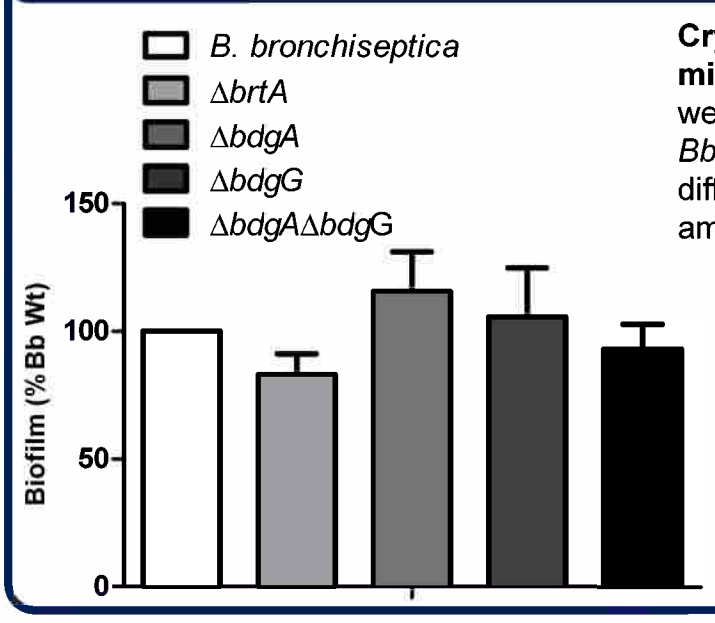


Fig 7. BdcG DGC activity was assessed using two different methodologies. **a.** BdcG heterologous expression on the *Ps. Fluorescens* Δ4DGC strain. * indicates a significant difference between strains ($p<0.05$) . **b.** Quantitative measurements of cellular c-di-GMP in the BATCH context.

8. BdcA and BdcG role on biofilm formation



Crystal violet based microtiter assay. Results were normalized against *Bb* WT. No significant difference were found among strains.

Second messengers group at IBBM. Web page

Results and conclusions

Using a bioinformatic methodology we have successfully predicted the interaction between LapD an BdcG. Besides LapD-BdcG we have also proven that BdcA physically interacts with LapD. Despite several efforts to prove if BdcG is an active DGC we were not able to find an experimental condition to show that. We speculate that BdcG might need an activation signal that is absent on our tested culture conditions. Biofilm formation assays with simple and double mutants show no difference against *B. bronchiseptica* WT. This result suggests that there might be function redundancy among DGCs. Besides BdcA and BdcG there might be other factors acting to regulate this phenotype. This is in concordance with recent findings that show that cdG regulation network is an intricated process and that more than one DGC could take part on the regulation of a single process.

1. Newell et al. 2011. Systematic analysis of diguanylate cyclases that promote biofilm formation by *Pseudomonas fluorescens* Pf0-1.
2. Ambrosis et al. 2016. Homologs of the LapD-LapG c-di-GMP Effector System Control Biofilm Formation by *Bordetella bronchiseptica*.
3. Sisti et al. 2013. Cyclic-di-GMP signaling regulates motility and biofilm formation in *Bordetella bronchiseptica*.
4. Kelly et al. 2015. The Phyre2 web portal for protein modeling, prediction, and analysis.
5. Dahlstrom et al. 2015. Contribution of Physical Interactions to Signaling Specificity between a Diguanylate Cyclase and Its Effector.

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