

## **Supplementary material**

### **HqiA, a novel quorum-quenching enzyme which expands the AHL lactonase family**

Marta Torres<sup>a,b</sup>, Stéphane Uroz<sup>c</sup>, Rafael Salto<sup>d</sup>, Laure Fauchery<sup>c</sup>, Emilia Quesada<sup>a,b</sup>,  
Inmaculada Llamas<sup>a,b\*</sup>

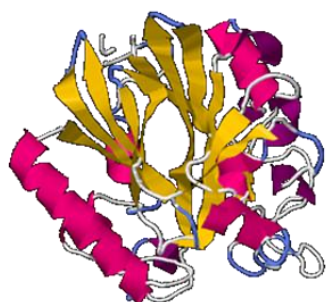
<sup>a</sup>Department of Microbiology, Faculty of Pharmacy, University of Granada, Granada, Spain.

<sup>b</sup>Institute of Biotechnology, Biomedical Research Center (CIBM), University of Granada, Granada, Spain.

<sup>c</sup>UMR 1136 INRA-Université de Lorraine Interactions Arbres-Microorganismes, Centre INRA de Nancy, Champenoux, France.

<sup>d</sup>Department of Biochemistry and Molecular Biology II, Faculty of Pharmacy, University of Granada, Granada, Spain.

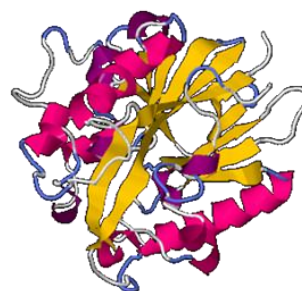
\*Author for correspondence: Inmaculada Llamas. Department of Microbiology, Faculty of Pharmacy, Campus de la Cartuja s/n, Granada, Spain. 958243871. illamas@ugr.es



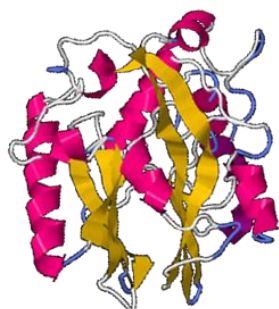
Lactonase AiiA  
*Bacillus thuringiensis*



Lactonase AiiB  
*Agrobacterium tumefaciens*



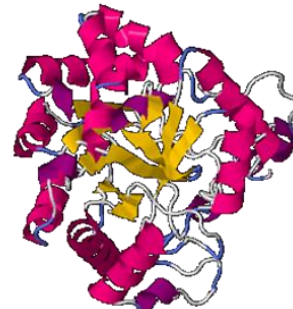
Lactonase AttM  
*Agrobacterium tumefaciens*



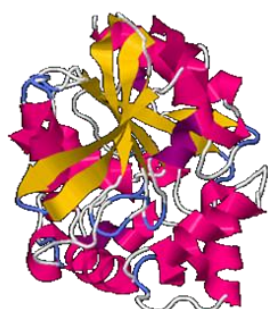
Lactonase AhIK  
*Klebsiella pneumoniae*



Lactonase QlcA  
homology with *Acidobacterium* sp.



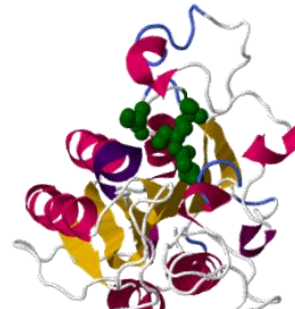
Lactonase QsdA  
*Rhodococcus erythropolis*



Lactonase AidH  
*Ochrobactrum* sp.

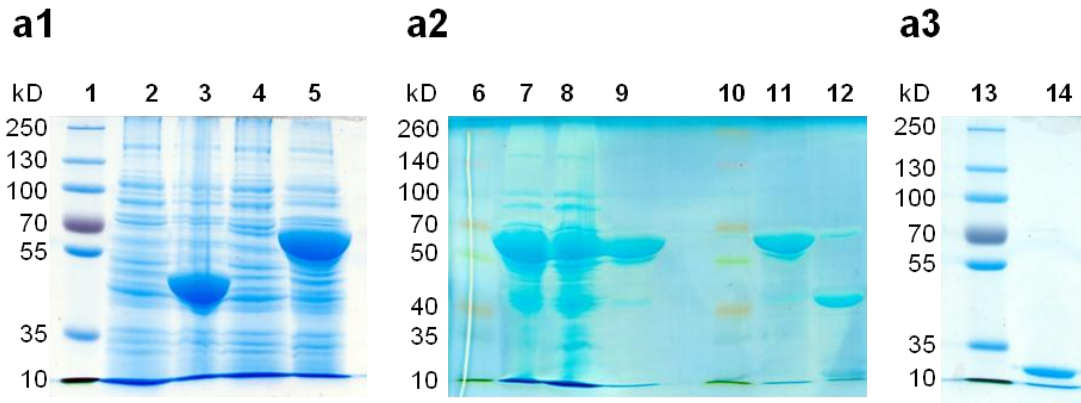


Lactonase HqiA  
homology with *Hyphomonas* sp.

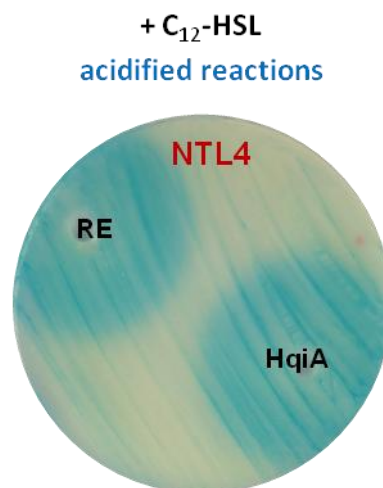


Lactonase HqiA  
showing catalytic site

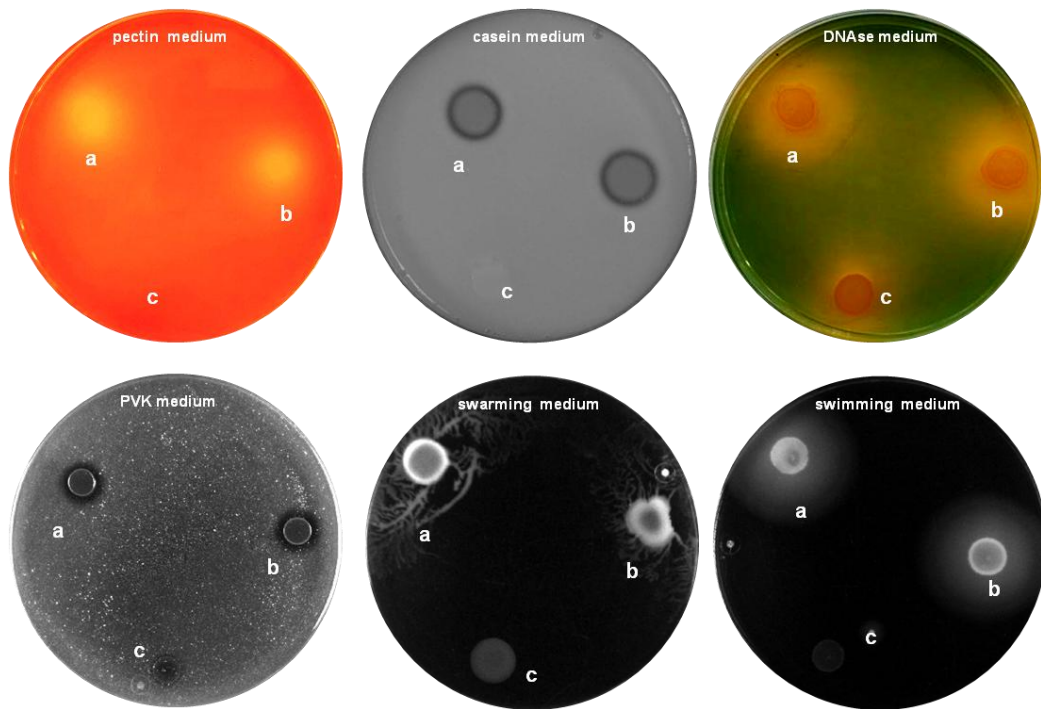
**Supplementary Figure S1. Protein structure of prototypic lactonases and HqiA.** HqiA showing the conserved catalytic domains D38, K144 and C147 (highlighted in green) of the CSHase family.



**Supplementary Figure S2. HqiA protein expression and purification.** Protein electrophoresis of MBP-HqiA expression (a1). Lane 1: protein molecular weight marker, lanes 2 and 3: non-induced and induced extracts from empty vector transformed bacteria (coding for a 42.2 kD protein), lanes 4 and 5: non-induced and induced extracts of pMAL-c2TEV-ORF29 transformed bacteria (coding for a 68.7 kD fusion protein). MBP-HqiA purification and proteolytic processing (a2). Lanes 6 and 10: protein molecular weight marker, lane 7: crude extracts, lane 8: soluble extracts, lanes 9 and 11: affinity chromatography fractions, lane 12: TEV protease processed fusion protein. Ion exchange chromatography (a3). Lane 13: protein molecular weight marker, lane 14: ion exchange chromatography eluted sample.



**Supplementary Figure S3. Lactone ring closure assay.** Detection of AHLs in the reaction buffer (RE) and HqiA protein with C<sub>12</sub>-HSL after acidification using the biosensor *Agrobacterium tumefaciens* NTL4 (pZLR4).



**Supplementary Figure S4. Influence of *hqiA* in some QS-regulated phenotypes of *P. carotovorum*.** *P. carotovorum* CECT 225<sup>T</sup> (1), *P. carotovorum* (pME6010) (2) and *P. carotovorum* (pME6010::*hqiA*) (3) tested in pectin, casein, DNase, PVK (alkaline phosphatase), swarming and swimming media.