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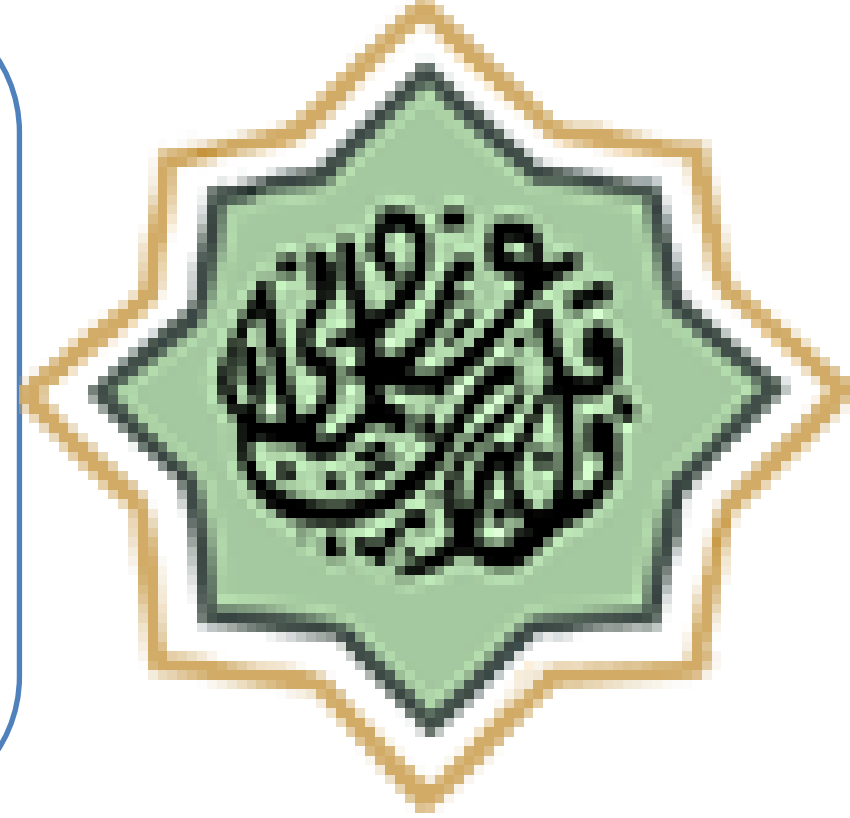
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Shewanella woodyi MS32: A new luminescent biosensor able to detect C8-AHL

Mahmoud HAYEK, Claudine BARAQUET, Yves BLACHE and Maëlle MOLMERET



Abstract

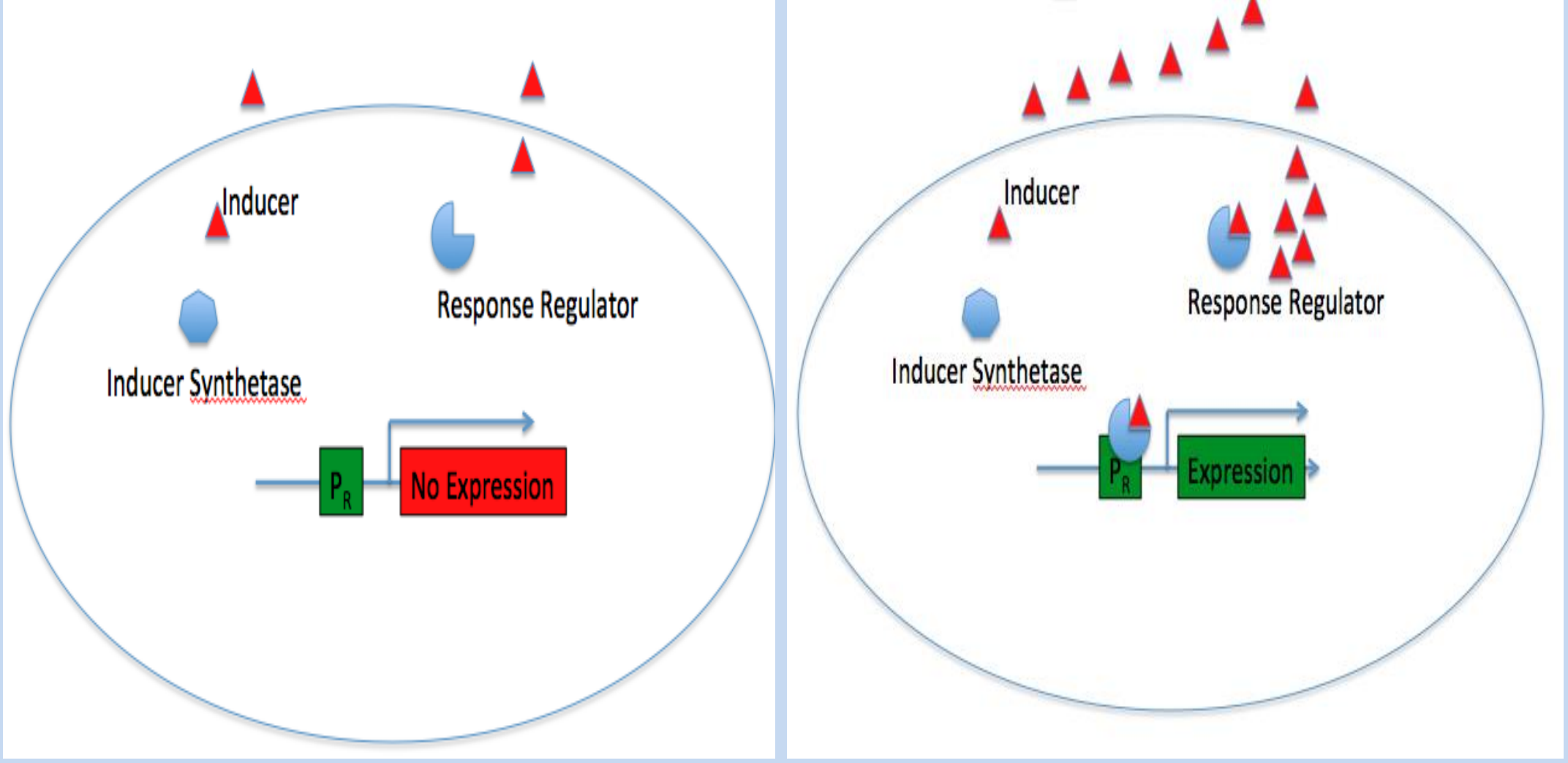
Quorum sensing (QS) or cell-to-cell communication is a process by which bacteria produce and detect signal molecules and thereby coordinate their behavior in a cell-density dependent manner. The sequenced genome of the marine bioluminescent strain *S. woodyi* MS32 contains genes coding for the AHL QS system. The goal of this study is to identify the AHL QS communication system present in this bacterium and to understand its role in biofilm formation. The objective is also to develop a new marine QS biosensor from this strain.

By coupling bacterial biosensor assays and high resolution liquid chromatography mass spectrometry (LCMS), we showed that *S. woodyi* synthesized N-octanoyl-L-homoserine lactone (C8-AHL). We studied the effect of a wide range of AHLs on the *S. woodyi* luminescence and only C8-AHL molecule induces luminescence.

A mutation of the AHL system genes, the *luxI* synthase or the *luxR* receptor makes *S. woodyi* non-luminescent but surprisingly *S. woodyi* mutant and wild type have the same adhesion and biofilm formation pattern. On the other hand, the QS mutation reduces phagocytosis of *S. woodyi* by amoeba eukaryotic cells.

Introduction

Bacterial communication by AHL quorum sensing

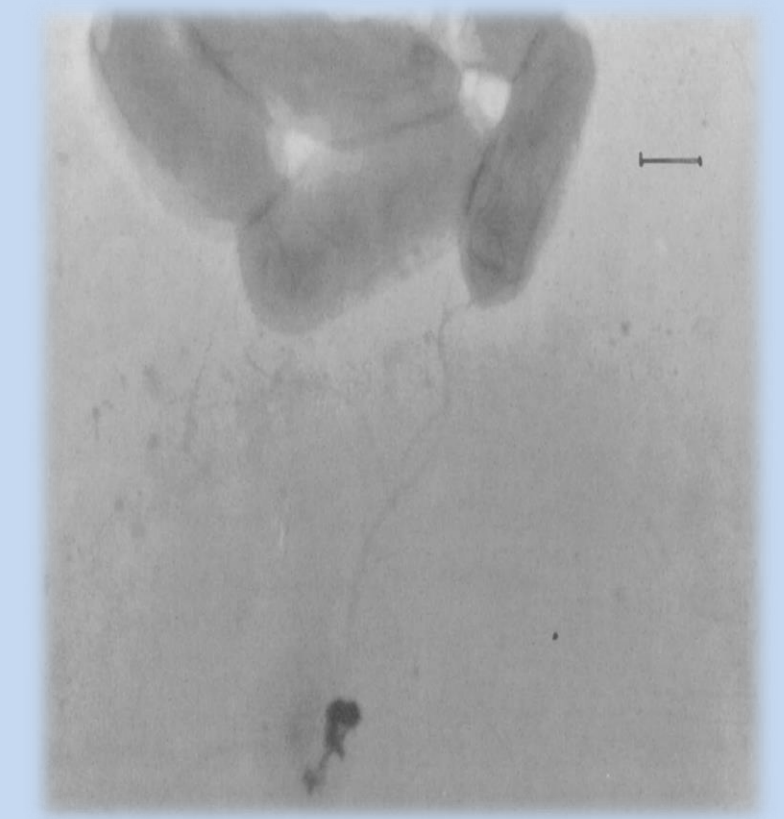


Low cell density

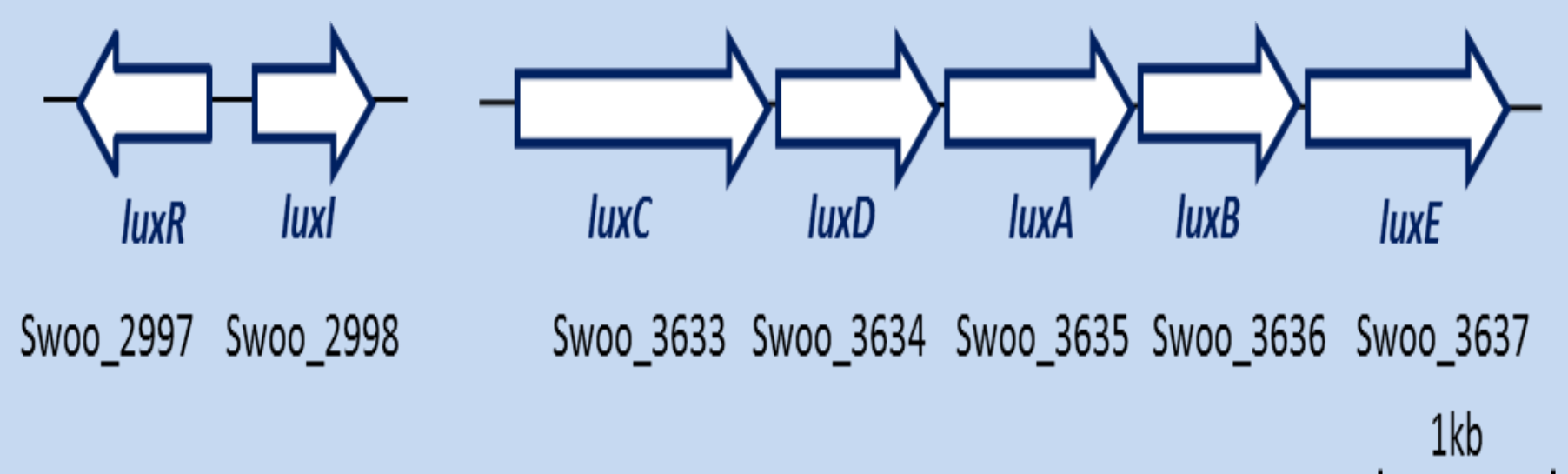
High cell density

Shewanella woodyi MS32

Gram-negative marine bacteria
Luminescent
Single polar flagellum
Biofilm formation

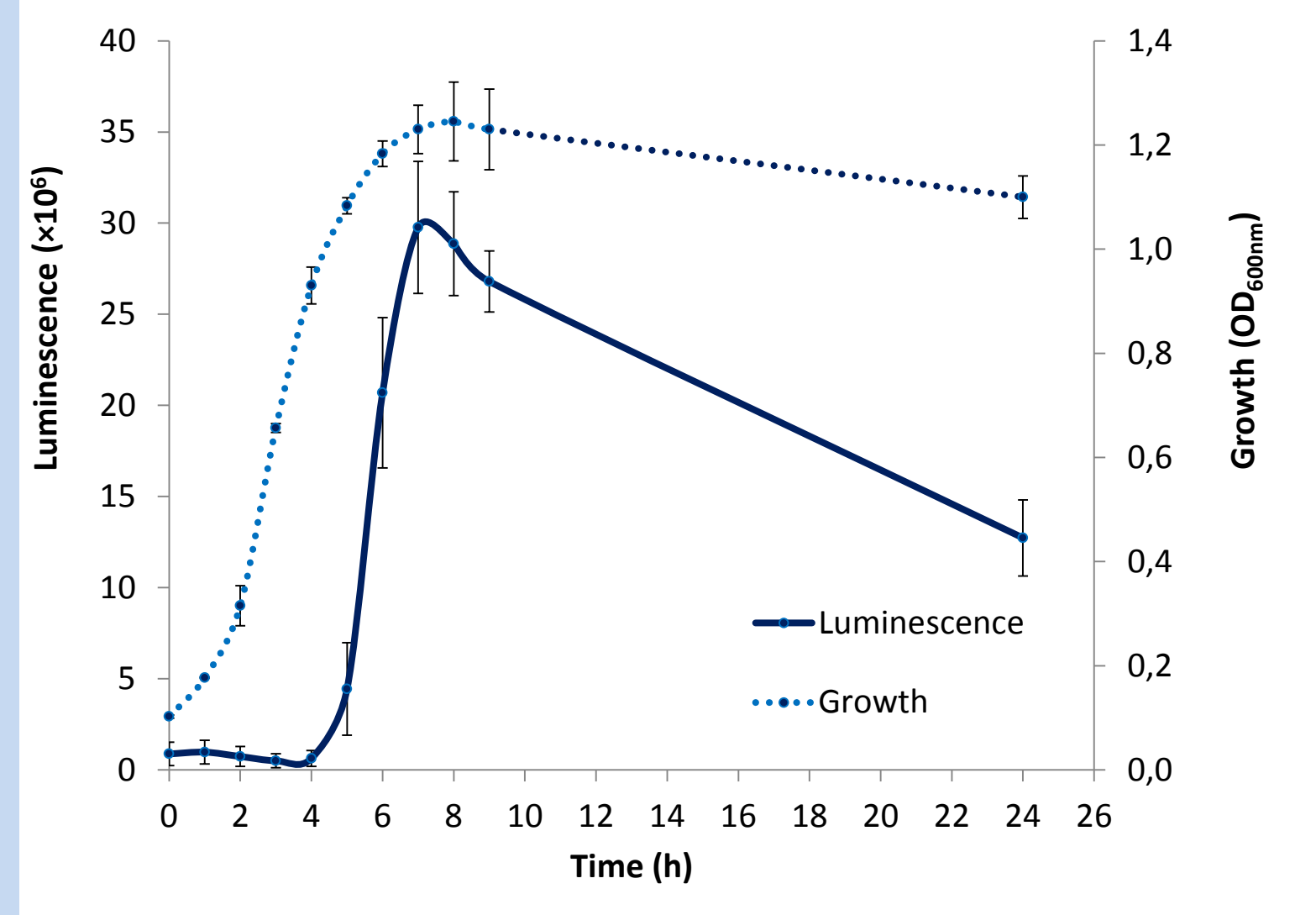


Quorum sensing genes:
luxI (Swoo_2998)
luxR (Swoo_2997)

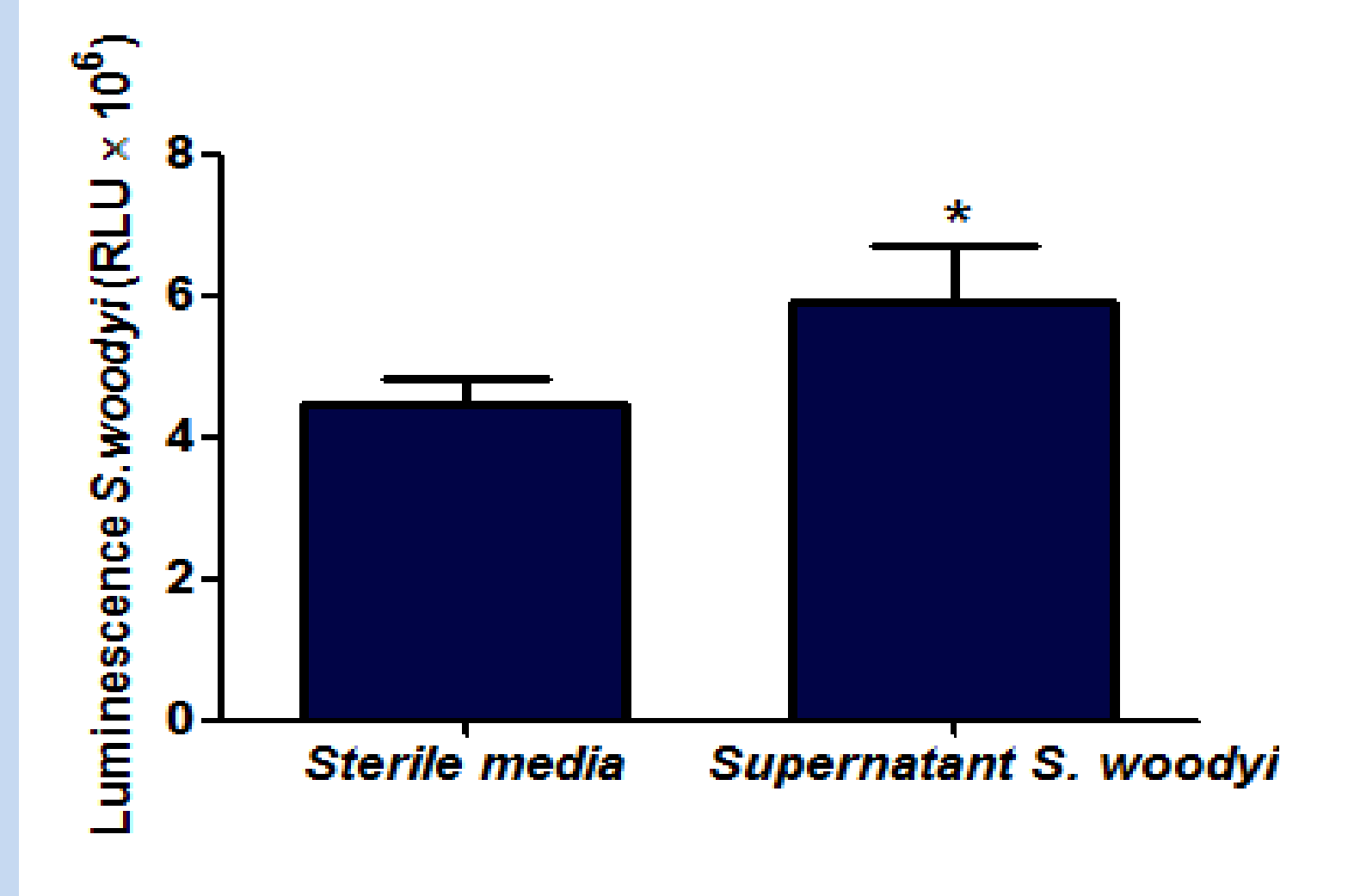


S. woodyi is a luminescent strain

Luminescence of *S. woodyi* is directly correlated with growth

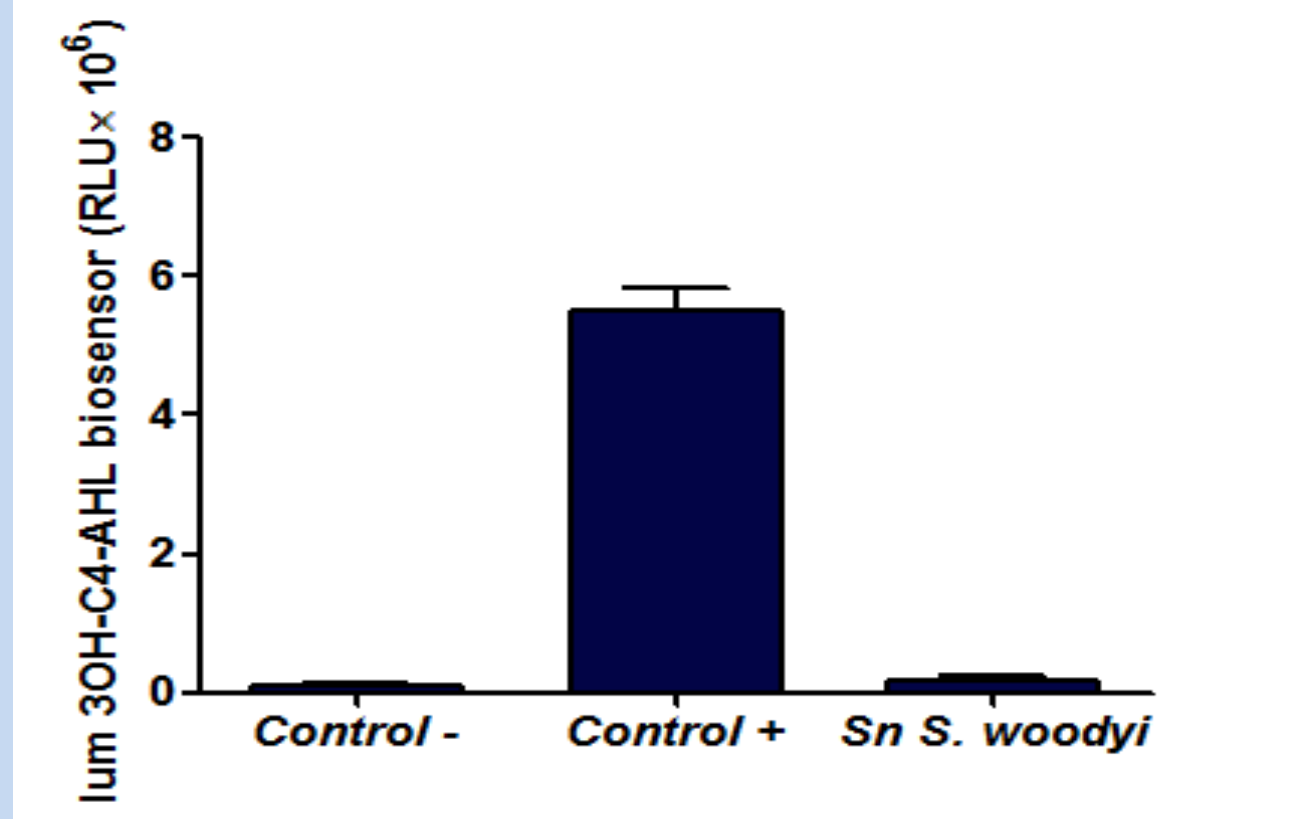


S. woodyi has an active QS system

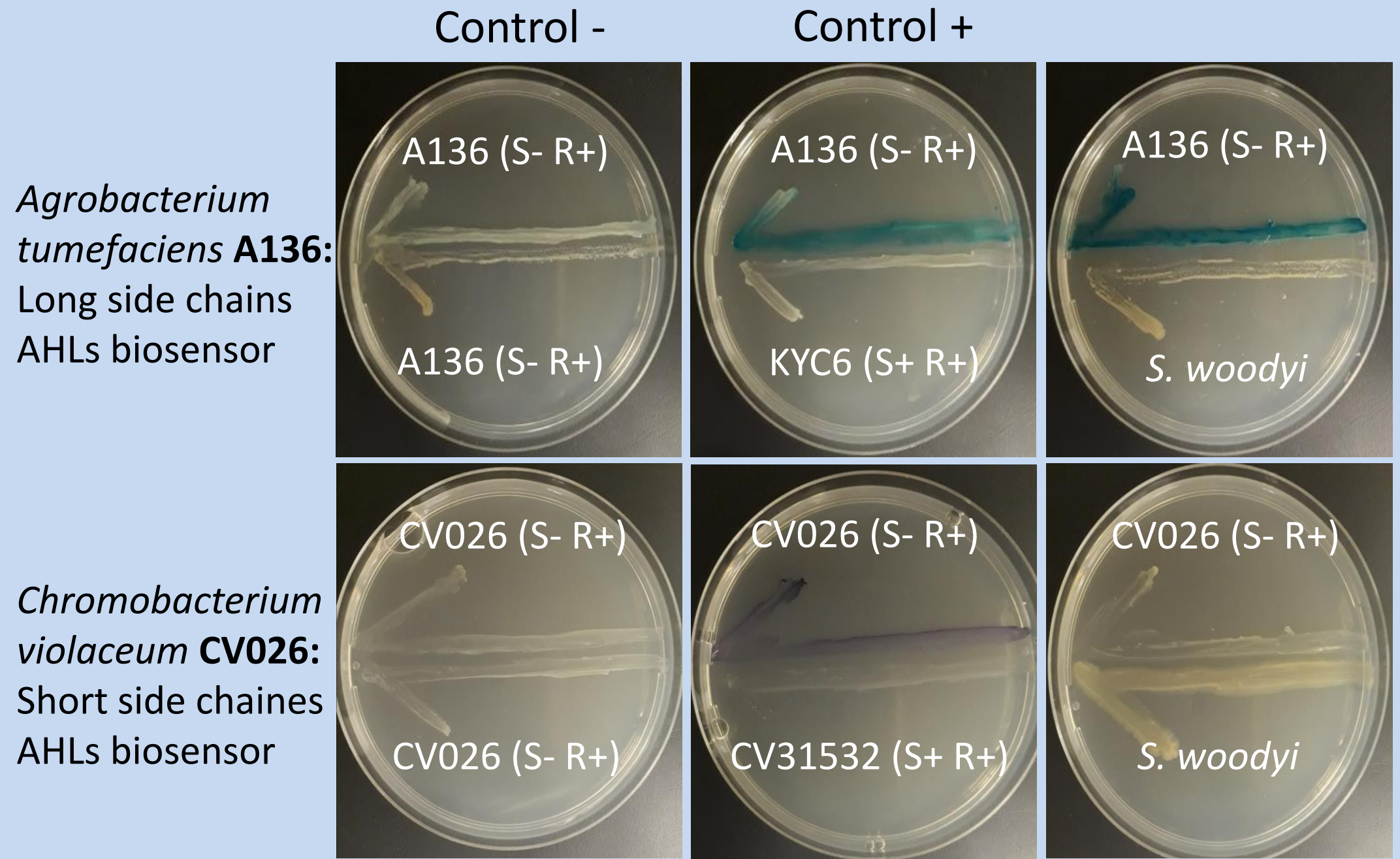


S. woodyi synthesizes the N-Octanoyl-DL-homoserine lactone (C8-AHL)

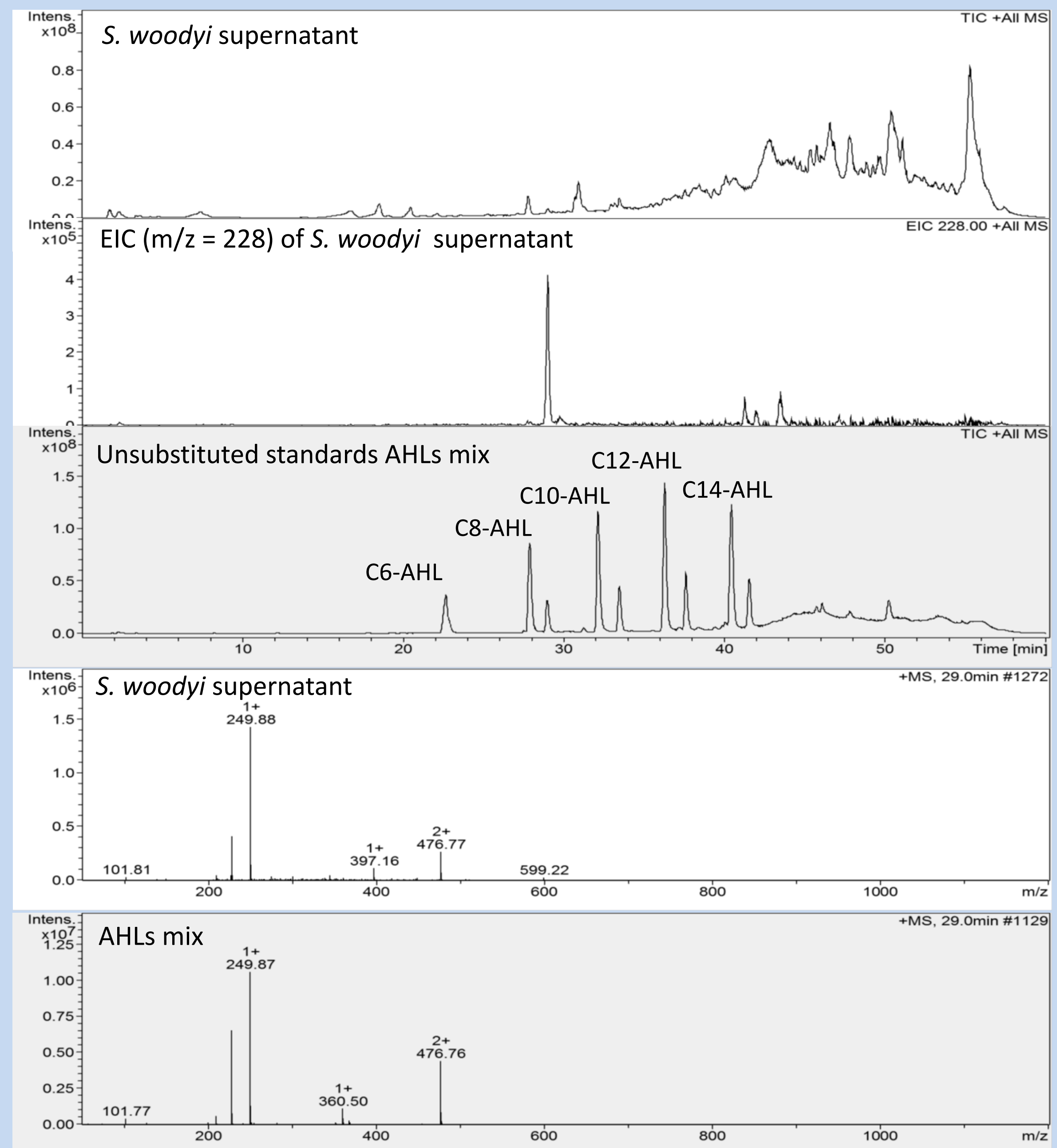
S. woodyi does not synthesize the 3OH-C4-AHL



S. woodyi synthesizes one or more long side chain AHL molecules



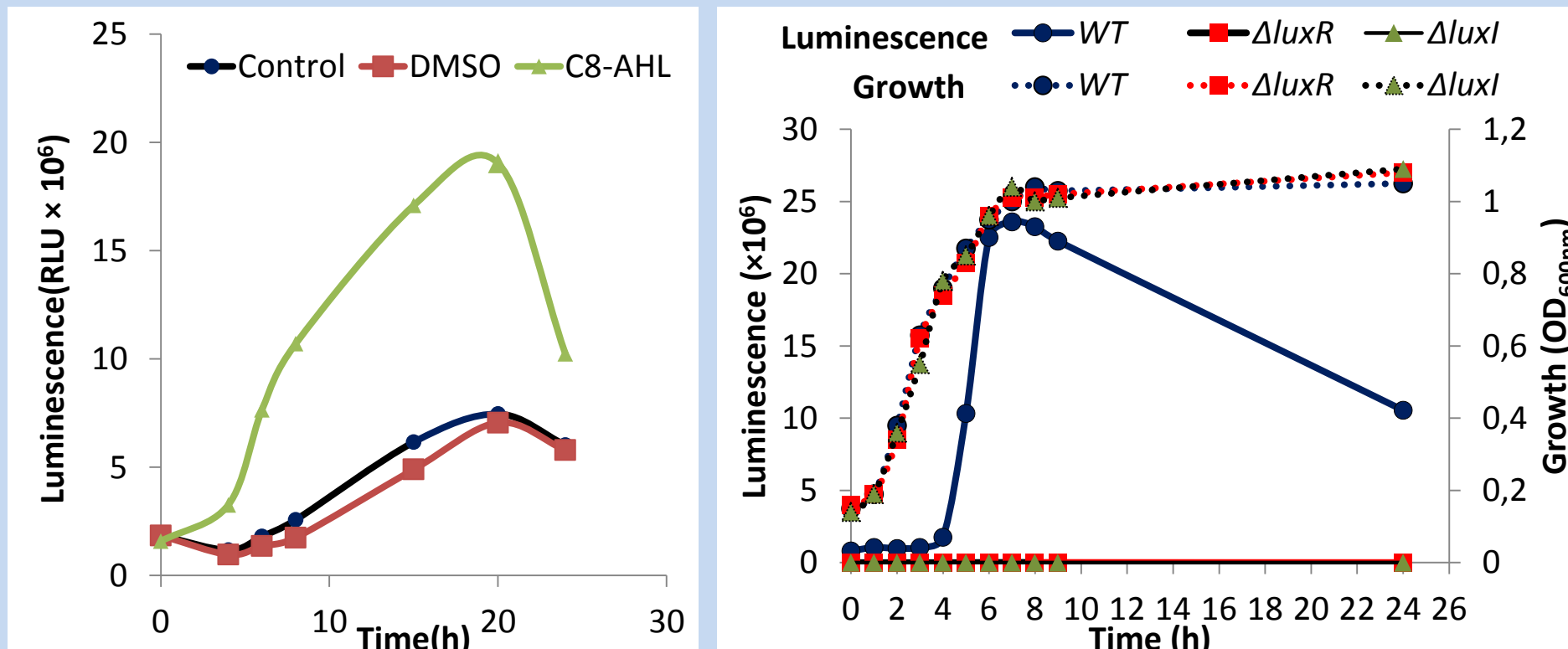
Detection of C8-AHL by LCMS in *S. woodyi* supernatant



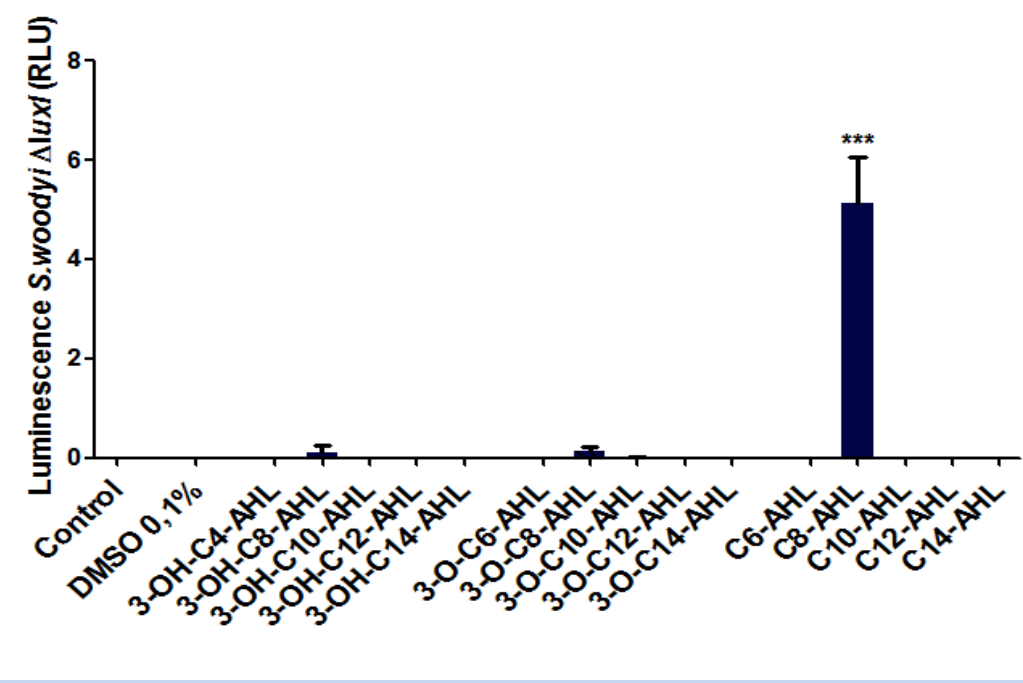
The luminescence of *S. woodyi* is regulated by QS system

C8-AHL induces *S. woodyi* luminescence

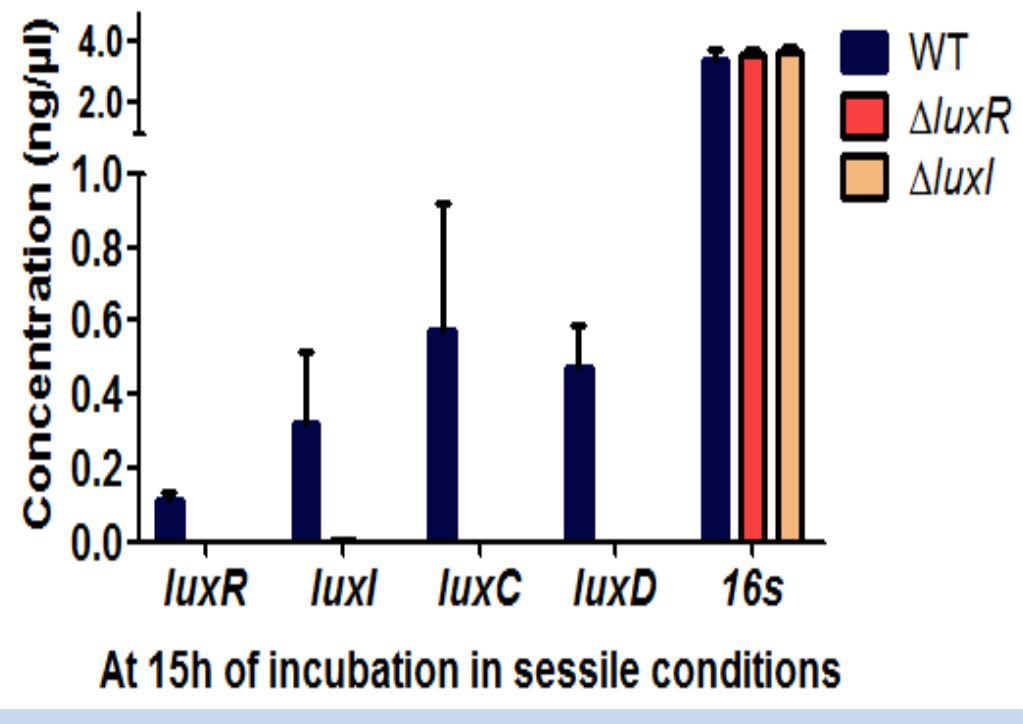
luxR or *luxI* mutation makes *S. woodyi* non luminescent



Only C8-AHL can induce luminescence in a *S. woodyi* $\Delta luxI$ background

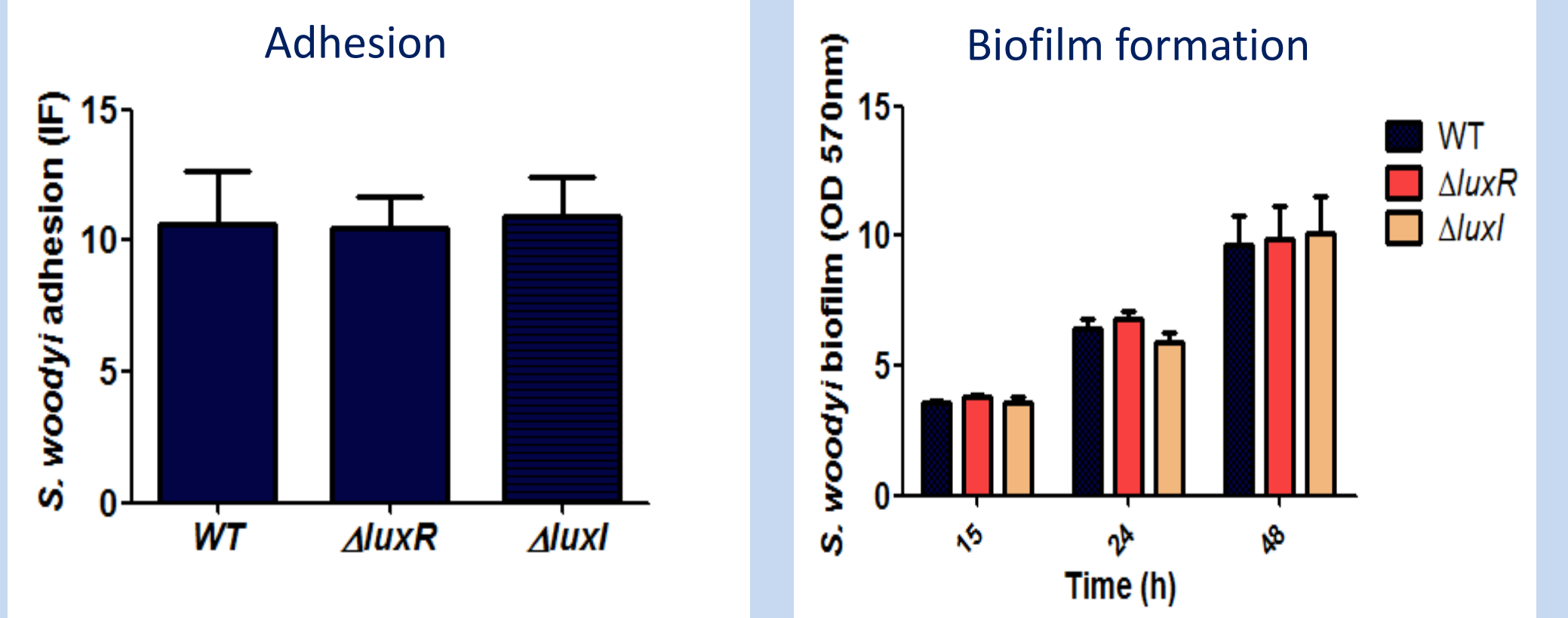


luxR or *luxI* mutants abolish QS genes expression

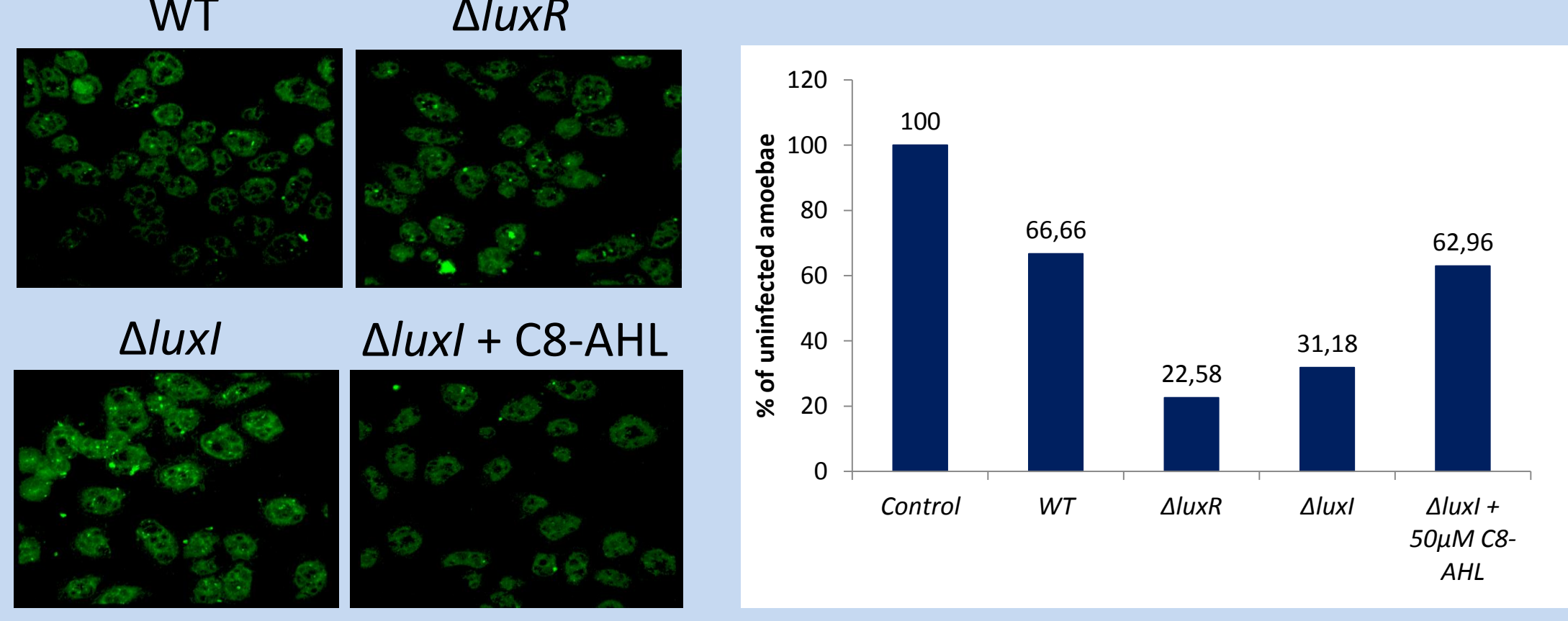


Adhesion and biofilm formation do not seem to be regulated by QS in *S. woodyi*

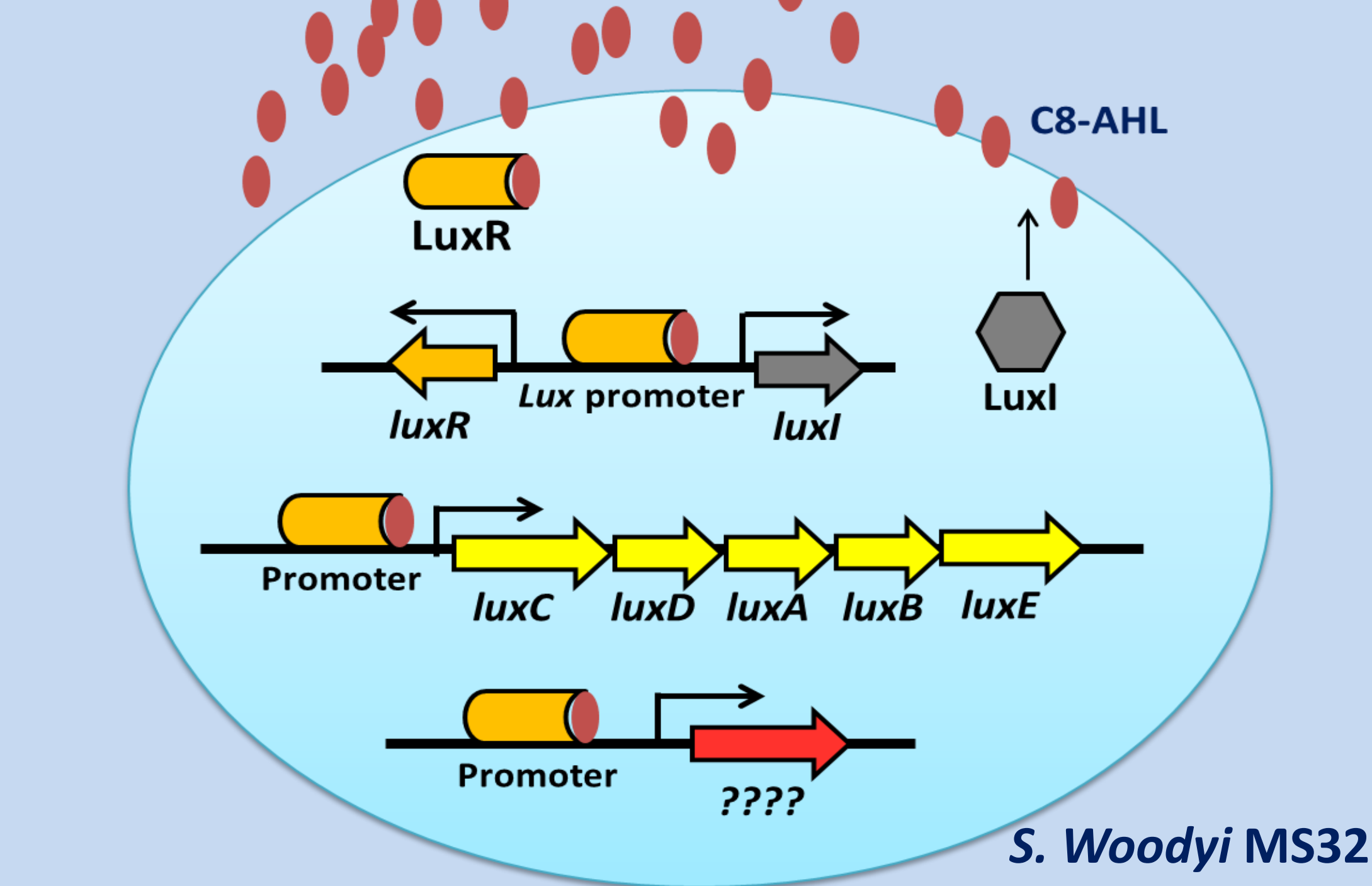
S. woodyi WT, $\Delta luxR$ or $\Delta luxI$ have the same adhesion and biofilm formation pattern



Mutation of QS genes appears to reduce phagocytosis of *S. woodyi* by amoeba eukaryotic cells



Conclusion



- ✓ Luminescence of *S. woodyi* is under the control of the AHL QS system
- ✓ *S. woodyi* produces and responds to C8-AHL : proof of concept to use *S. woodyi* as a biosensor to screen the synthesis of C8-AHL by other bacteria
- ✓ Exception to the general rule, QS in *Shewanella woodyi* is not involved in adhesion and biofilm formation
- ✓ Mutation of QS genes reduces phagocytosis of *S. woodyi* by amoeba eukaryotic cells