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Characterization of *Shewanella woodyi* Quorum Sensing systems

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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. Two main QS systems can be distinguished: the acylhomoserine lactone (AHL) and the autoinducer-2 (AI-2). The sequenced genome of the marine bioluminescent *Shewanella woodyi* MS32 contains genes coding for these two main QS systems. This work aims at identifying the QS communication systems present in this bacterium and understanding its role in luminescence production and biofilm formation. The objective is also to find molecules that interfere with QS processes to inhibit adhesion and biofilm formation.

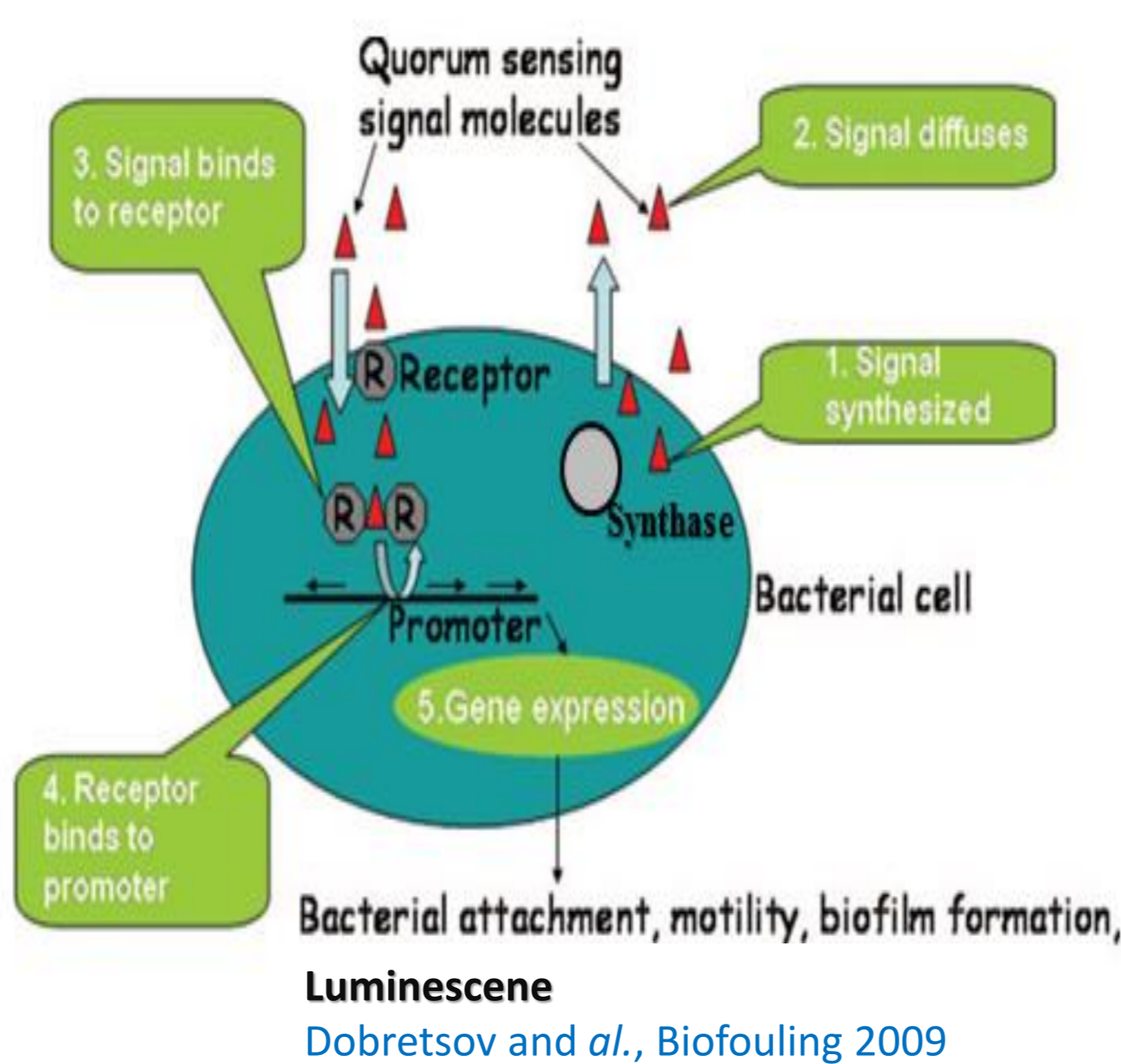
By coupling bacterial biosensor assays and high resolution liquid chromatography mass spectrometry (LCMS), we showed that *S. woodyi* synthesized both a N-octanoyl-l-homoserine lactone (C8-HSL) and the AI2 QS molecule. However, only HSL molecules influence the luminescence of *S. woodyi* in particular oxo and hydroxy derivatives.

Several commercial molecules, identified for their inhibitory effect on the AI2 QS system of *Vibrio harveyi* inhibited the luminescence of *S. woodyi* and reduced adhesion and biofilm formation, which led to the conclusion that AI-2 is not likely used as a quorum sensing signal and that LuxS is involved in the activated methyl cycle (AMC) in *S. woodyi*.

This study is the first documentation that characterizes the QS system in *S. woodyi*.

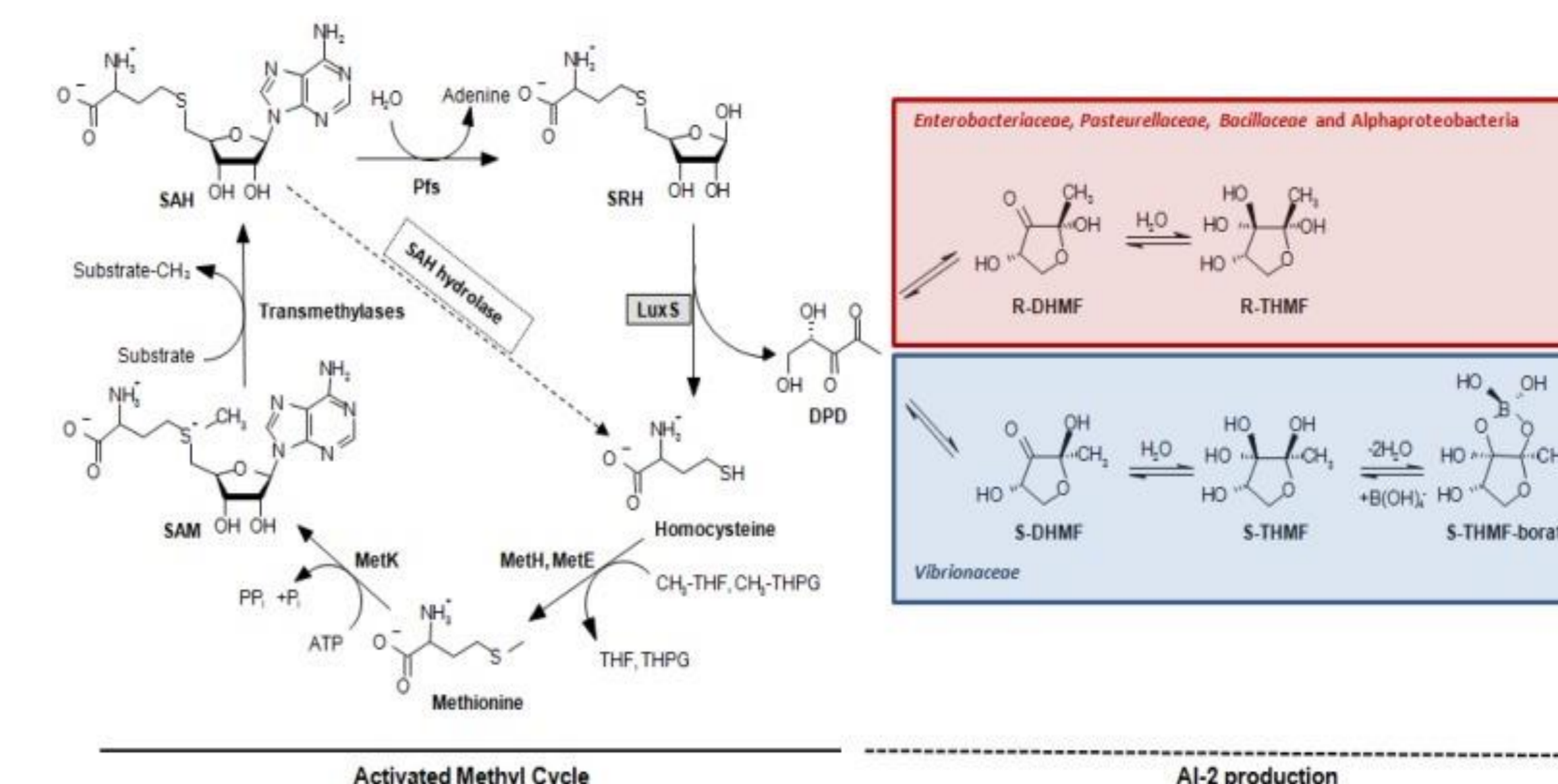
Introduction

Bacterial communication by quorum sensing (QS)



QS system	Signal molecule	Synthase/receptor
AHL system	Acylhomoserin lactone (AHL)	LuxI / LuxR
AI2 system	Autoinducer 2 (AI2)	LuxS / LuxPQ

Activated methyl cycle and AI2 biosynthesis



Rezzonico and Duffy, BMC Microbiology 2008

Shewanella woodyi MS32



Gram-negative marine bacteria
Luminescent
Single polar flagellum
Biofilm formation

Quorum sensing genes:
luxI (Swoo_2998)
luxR (Swoo_2997)
luxS (Swoo_3618)

Objectives

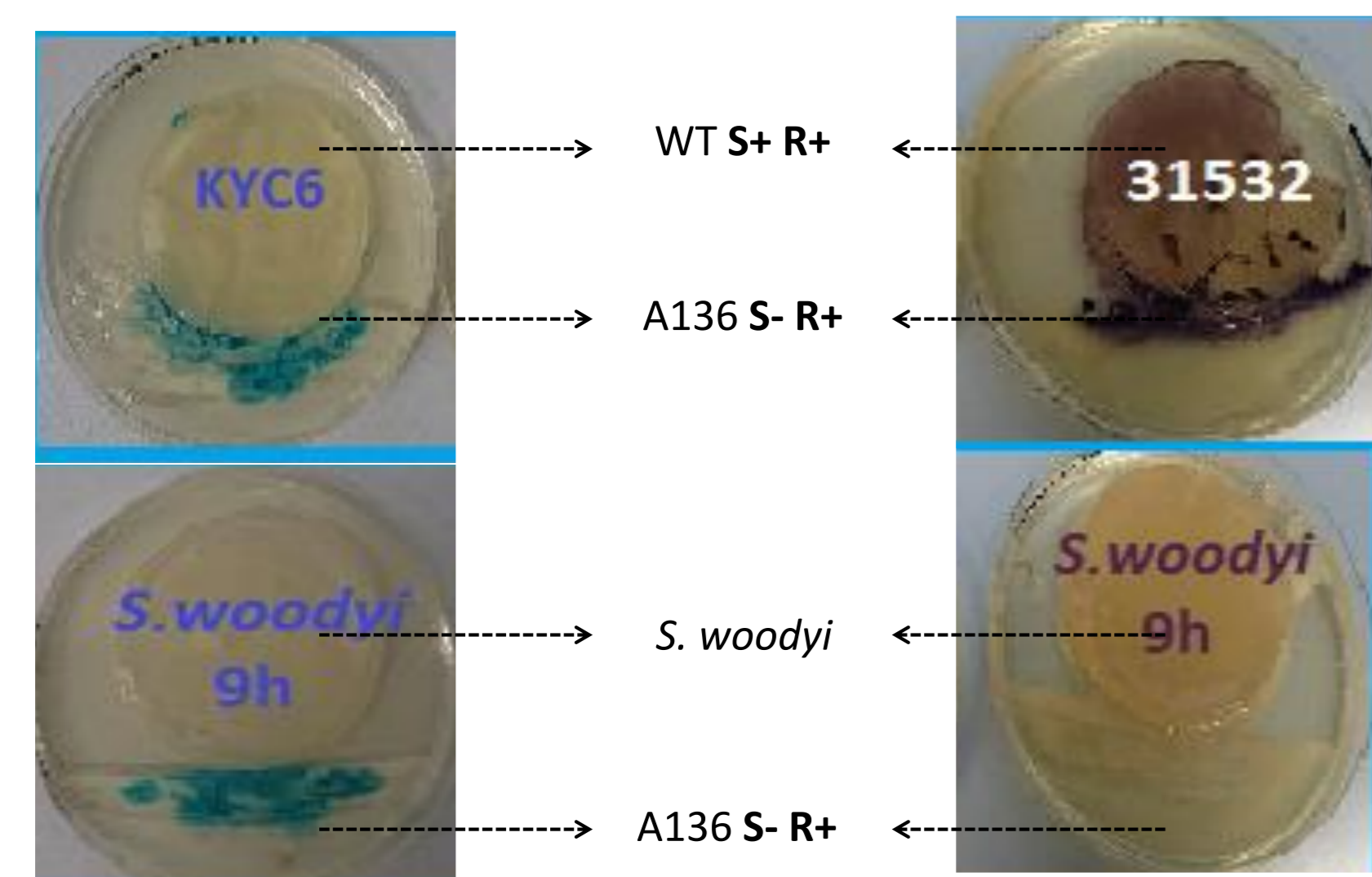
- To identify the QS communication systems
- To understand the role of QS in biofilm formation
- To find molecules targeting QS pathways to inhibit adhesion and biofilm formation

S. woodyi synthesizes only one AHL : the C8-HSL

- S. woodyi* produces long side chains AHLs

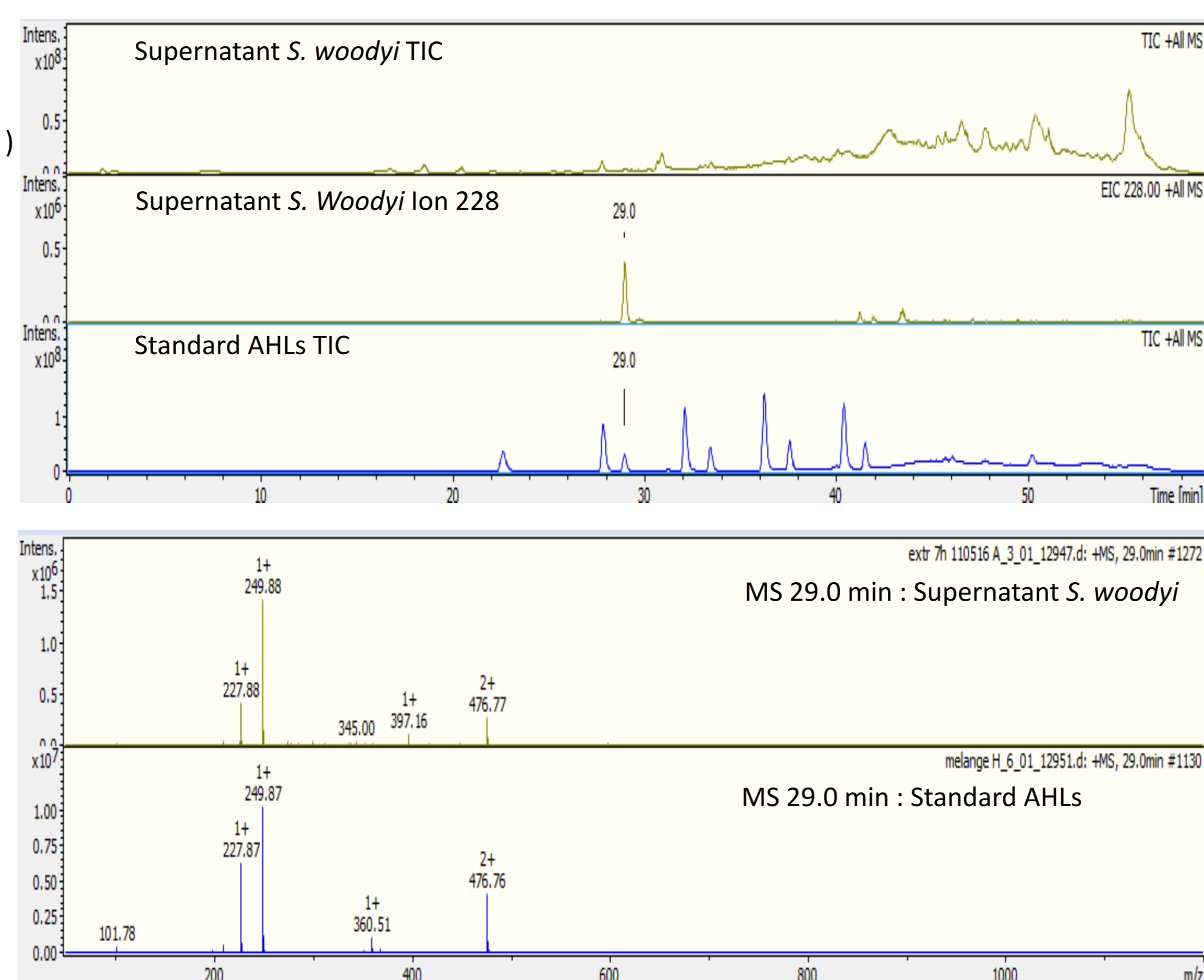
Long side chains AHLs biosensor
(*Agrobacterium tumefaciens* A 136)

Short side chains AHLs biosensor
(*Chromobacterium violaceum* CV 026)

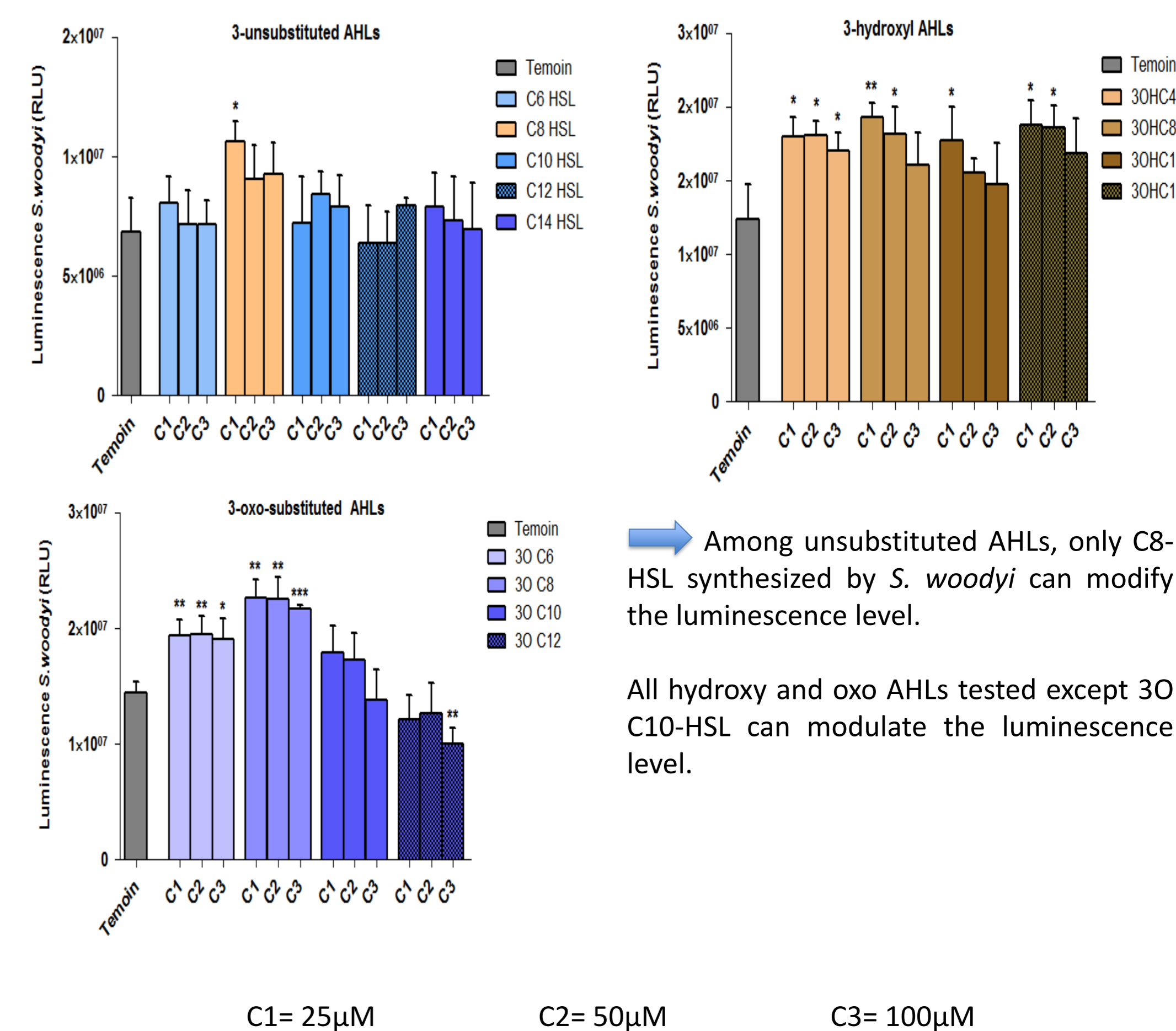


S+/- = presence/absence of the synthase gene (*luxI*)
R+/- = presence/absence of the receptor gene (*luxR*)

- Supernatant of *S. woodyi* contains C8-HSL



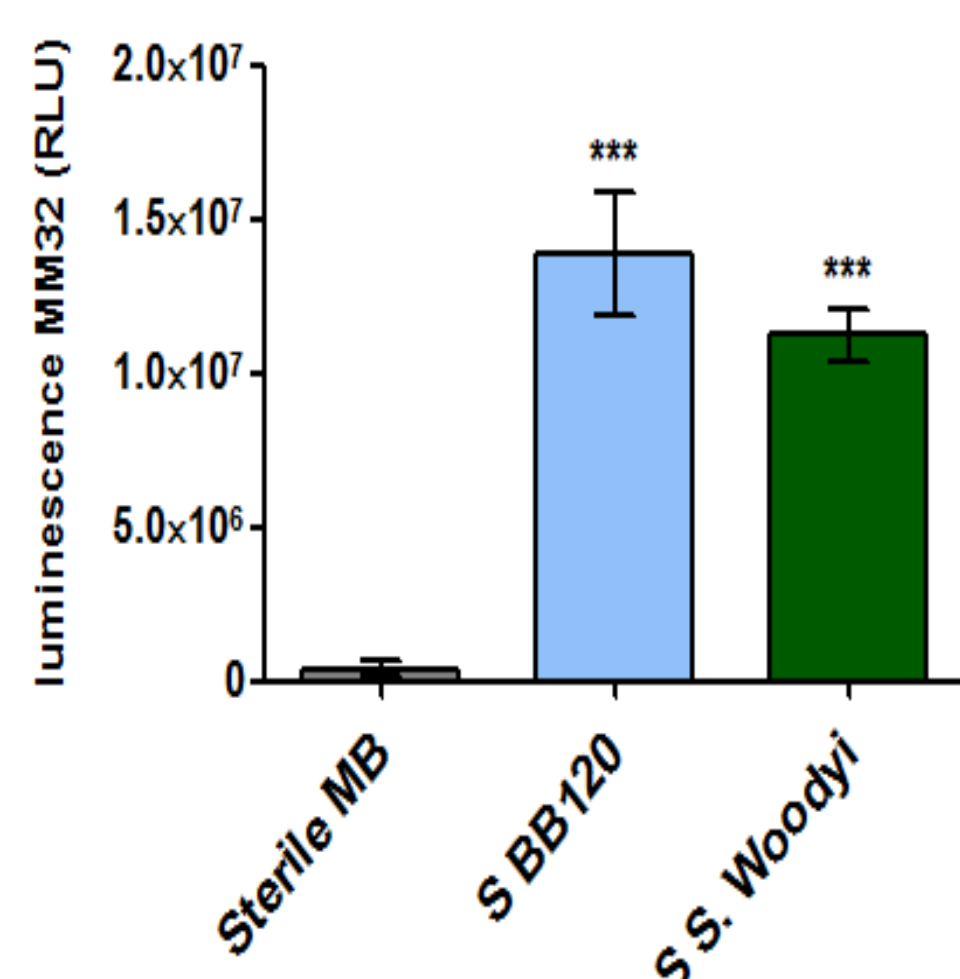
Various AHLs modulate *S. woodyi* luminescence



Among unsubstituted AHLs, only C8-HSL synthesized by *S. woodyi* can modify the luminescence level.

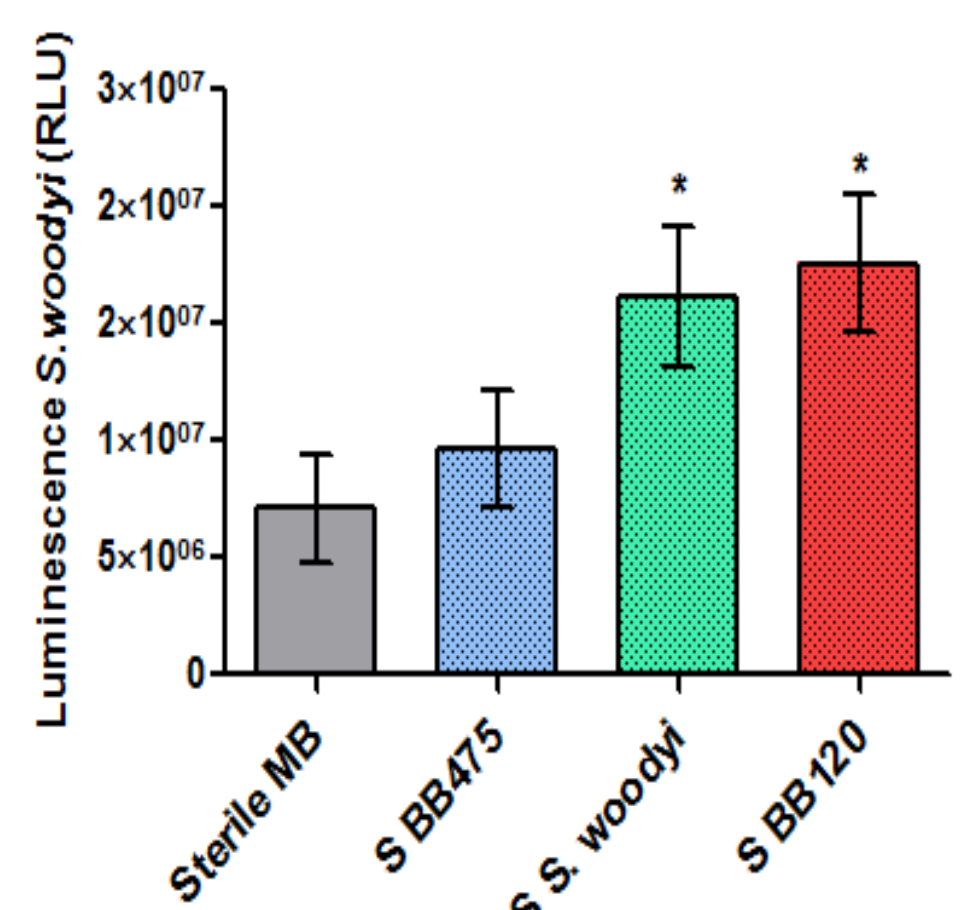
All hydroxy and oxo AHLs tested except 3O C10-HSL can modulate the luminescence level.

S. woodyi produces AI2, however luminescence is under the control of the AHL QS system.



Bioluminescence induction in the AI2 biosensor *Vibrio harveyi* MM32 after addition of Marine Broth (MB, negative control), culture supernatant of *V. harveyi* BB475 as a source of AI2, culture supernatant of *S. woodyi* or culture supernatant of *V. harveyi* WT BB120 as a source of exogenous AI2 (positive control) or culture supernatant of *S. woodyi*.

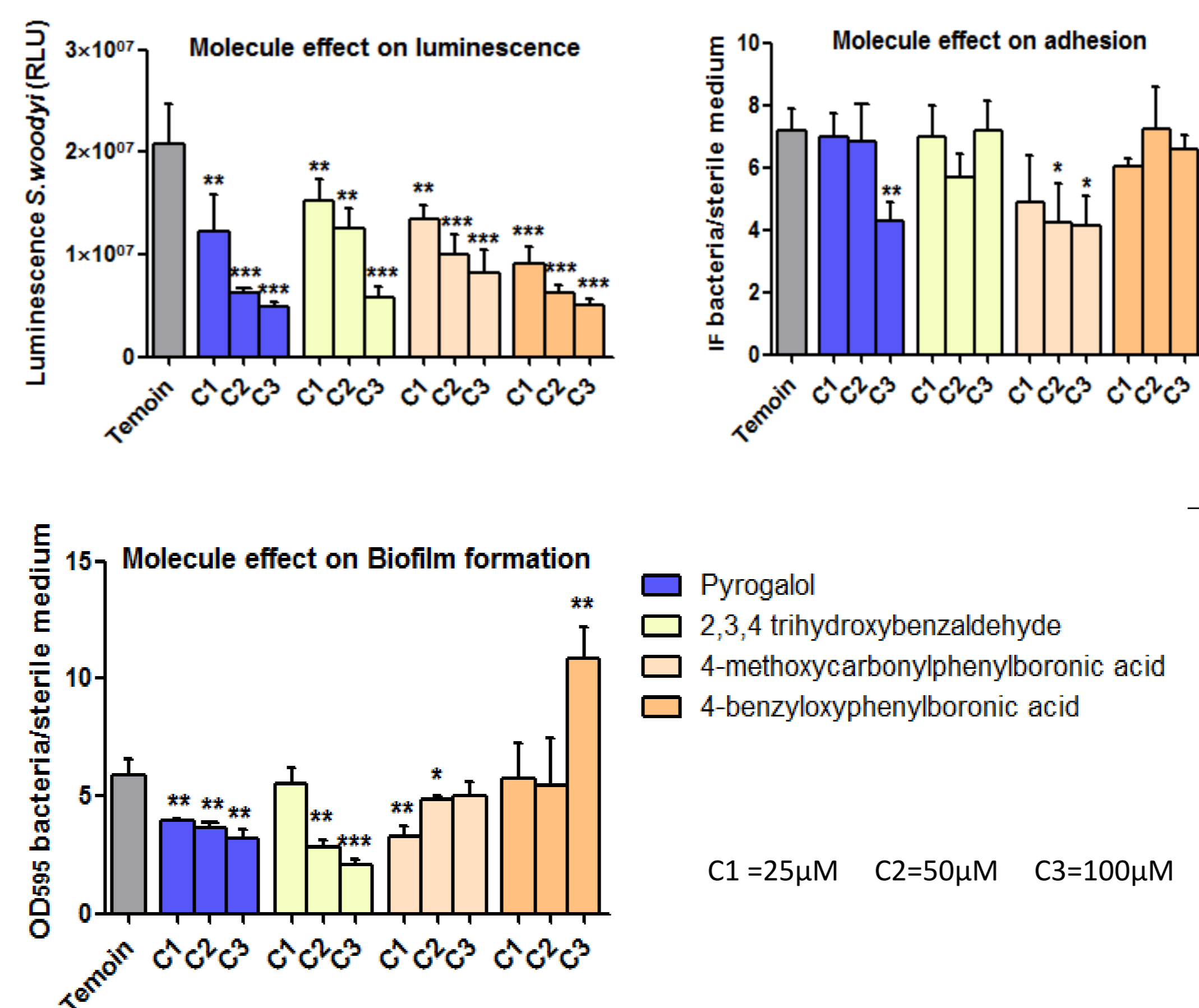
→ *S. woodyi* produces AI2



Bioluminescence induction in *S. woodyi* after addition of Marine Broth (MB, negative control), culture supernatant of *V. harveyi* BB475 as a source of AI2, culture supernatant of *S. woodyi* or culture supernatant of *V. harveyi* WT BB120 as a source of exogenous AI2 and 3OH C4-HSL.

→ Luminescence of *S. woodyi* is mediated by the AHL QS system and is not under the control of the AI2 QS system. This observation is in good agreement with the absence of the *luxPQ* genes in the genome of *S. woodyi*.

Effect of commercial molecules on QS-related cellular functions: luminescence, adhesion and biofilm formation



→ All tested molecules that inhibit QS also inhibit biofilm formation in *S. woodyi* except the 4-benzoyloxyphenylboronic acid, which increases biofilm formation at a concentration = 100μM

Conclusion

- ✓ Luminescence of *S. woodyi* is under the control of the AHL QS system. *S. woodyi* produces C8-HSL but responds to other AHLs molecules, in particular oxo and hydroxy derivatives.
- ✓ *S. woodyi* produces AI2 but AI2 does not seem to be involved in luminescence.
- ✓ QS inhibition is related to biofilm/adhesion inhibition in *S. woodyi*: proof of concept to use *S. woodyi* as a biosensor to screen anti-biofilm molecules.

References

- Dobretsov, S. et al. (2009), 'Mini-review: quorum sensing in the marine environment and its relationship to biofouling', Biofouling, 25(5): 413–427.
- Rezzonico, F. et al. (2012), 'Detection of AI-2 Receptors in Genomes of Enterobacteriaceae Suggests a Role of Type-2 Quorum Sensing in Closed Ecosystems', Sensors, 12(5): 6645–6665.