



## Anti-biofilm activity substances derived from coral symbiotic bacterial extract can be used to inhibit the biofouling

Yu Song (1), Zhonghua Cai (2), Guanghui Lin (1), Panqing Yin (2), Yongmin Lao (2), Hui Jin (2), Kezhen Ying (2), and Jin Zhou\* (2)

(1) Department of Earth System Science, Tsinghua University, China (songy17@mails.tsinghua.edu.cn), (2) Division of Ocean Science and Technology, Graduate School at Shenzhen, Tsinghua University, Shenzhen, China (zhou.jin@sz.tsinghua.edu.cn)

Marine biofouling, defined as the rapid and extensive growth of marine organisms on submerged inanimate and living surfaces, is a serious problem causing extensive material and economic costs worldwide. Over the last few decades, significant efforts have concentrated on mitigating biofouling in marine field, with a focus on non-toxic and sustainable strategies. Here, we explored the potential of applying anti-quorum sensing or anti-biofilm bacteria to control biofouling in a laboratory-scale system. About two hundreds strains were isolated from coral species (*Pocillopora damicornis*) and screened for their ability to inhibit QS using bio-reporter strain *Chromobacterium violaceum* CV12472. Approximately 15% of the isolates exhibited anti-QS activity against the indicator strain. Among them, a typical coral symbiotic bacterium, 12<sup>#</sup> (*Vibrio alginolyticus*) was isolated and demonstrate its anti-QS activity. Using crystal violet staining, we found that the extract of *V. alginolyticus* has the ability to reduced biofilm formation by 40% in model G<sup>-</sup> strain *Pseudomonas aeruginosa* PAO1. The confocal microscopy observations showed the 12<sup>#</sup> extract exhibited inhibited the biofilm related phenomenon (thickness and roughness) of *Pseudomonas aeruginosa*. After analyzed by nuclear magnetic resonance (NMR) and mass spectrometry, the bioactivity anti-biofilm molecule identified as rhodamine isothiocyanate. Furthermore, RT-PCR analyze showed that 12<sup>#</sup> led to a significant down-regulation of QS-regulatory genes and virulence-related genes, including *lasI*, *lasR*, *rhII*, *rhIR*, *pqsA* and *pqsR*. The whole genome sequence analysis reveals the presence of related rhodamine isothiocyanate synthase gene in strain 12<sup>#</sup>. We presume that the target bioactor may act as antagonists of bacterial quorum sensing by competing with QS signals for receptor binding. This work indicated that coral microbes sources play a vital role in delivering the novel agents candidates in anti-fouling field.