

Determination of Whether Quorum Quenching Is a Common Activity in Marine Bacteria by Analysis of Cultivable Bacteria and Metagenomic Sequences

Manuel Romero,^a Ana-Belen Martin-Cuadrado,^b and Ana Otero^a

Departamento de Microbiología y Parasitología, Facultad de Biología-CIBUS, Universidad de Santiago de Compostela, Santiago de Compostela, Spain,^a and Evolutionary Genomics Group, Departamento Producción Vegetal y Microbiología, Universidad Miguel Hernández, San Juan de Alicante, Spain^b

The abundance of quorum quenching (QQ) activity was evaluated in cultivable bacteria obtained from oceanic and estuarine seawater and compared with the frequency of QQ enzyme sequences in the available marine metagenomic collections. The possible role of the high QQ activity found among marine bacteria is discussed.

The low bacterial population encountered in the open sea and the low chemical stability of *N*-acyl-homoserine lactones (AHLs) in seawater have led to the suggestion that the AHL-mediated quorum sensing (QS) activity may be concentrated in specific microhabitats in the marine environment (3, 11) and that, therefore, the quorum quenching (QQ) processes would be expected to be less frequent in seawater. The presence of AHLs in marine particulate organic carbon has been recently confirmed, with more than 10% of the particle-associated bacteria being identified as AHL producers (10). Even higher QS activity values have been found for isolates from subtidal biofilms (31% [12]) and sponges (20 to 56% [14]), while a similar value was found for marine snow and microalgal isolates (9.3% [9]). Moreover, the addition of exogenous AHLs to incubations containing marine organic particles stimulated the activity of some key hydrolytic enzymes, suggesting a role of QS signals in the regulation of the degradation of particulate organic carbon (10). In a recent work, the importance of QQ processes in marine dense microbial communities was evidenced by the large amount of cultivable marine bacteria active against AHL signals that could be isolated from marine habitats such as sediments, biofilms, and the surface of the alga *Fucus vesiculosus* (18). These results indicate that QQ could be a usual strategy adopted in the marine environment to achieve competitive advantages at least in surfaces such as biofilms and eukaryotic niches. As an approximation to evaluate the ecological significance of QQ processes in the marine environment and to elucidate if QQ processes are as abundant in marine seawater pelagic microbial communities as previously described for coastal dense communities (18), we studied the presence of QQ activity among isolates from estuarine and open-ocean seawater. This activity was compared with the frequency of sequences homologous to known QQ enzymes in the available long-read marine metagenomic collections.

Quorum quenching activity among cultivable bacteria. In order to evaluate if QQ processes are as abundant among cultivable bacteria from marine seawater pelagic microbial communities as previously described for those isolated from marine surface-associated communities (18), 464 marine isolates were obtained from three different seawater samples using different culture media and temperatures and screened for detection of AHL-QQ activity using a bioassay based on *Chromobacterium violaceum* reporter strains as previously described (18). One of the samples was obtained from surface water at 10 m from the shore line in an

estuary (42°0.33'0.45.32"N, 8°0.53'0.08.23"W, Spain), and the other two samples were obtained from Atlantic Ocean water (42°0.17'0.07"N, 8°0.53'0.27"W) at 0- and 10-m depths. The density of cultivable bacteria in oceanic samples was around 1 order of magnitude lower than that in the seawater collected in the estuary, where the maximal number of CFU ml⁻¹ reached 4 × 10⁴ (see Fig. S1 in the supplemental material). Out of the 464 isolates, 85 were able to interfere with C₆- and C₁₀-HSL, which represents 18% of the strains studied, a percentage comparable to the percentage of QQ activity obtained for dense microbial communities from marine surfaces (14.4% [18]). As in the previous study (18), an important effect of the origin of the sample on the percentage of strains with QQ activity was observed: while strains from estuarine water presented a QQ activity of 2%, more than 20% of the strains isolated from oceanic samples were QQ active (28% and 22% for samples from 0- and 10-m depths, respectively; see Table S1 in the supplemental material).

The enzymatic degradation of AHL detected in the bioassay was confirmed by high-pressure liquid chromatography–mass spectrometry (HPLC-MS) as previously described (18). A shorter and a longer AHL (C₄- and C₁₂-HSL) were selected in order to check the spectrum of activity. All 85 strains selected as positives in the bioassay could completely eliminate C₁₂-HSL, which excludes the production of QS inhibitors by these positive strains. In contrast, only 4 out of the 85 strains tested were able to degrade C₄-HSL (see Fig. S2 in the supplemental material). Since short-chain AHLs are less stable than long-chain AHLs at high pH (24), the inability of these 81 strains to inactivate C₄-HSL confirms the enzymatic nature of the QQ activity. Moreover, the degradation capacity of the 4 strains generating complete degradation of the two AHLs was maintained in crude cell extracts (data not shown) obtained in phosphate-buffered saline (PBS), pH 6.5, as previously described (17), which rules out the possibility of an inacti-

Received 20 April 2012 Accepted 9 June 2012

Published ahead of print 15 June 2012

Address correspondence to Ana Otero, anamaria.otero@usc.es.

Supplemental material for this article may be found at <http://aem.asm.org/>.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AEM.01266-12

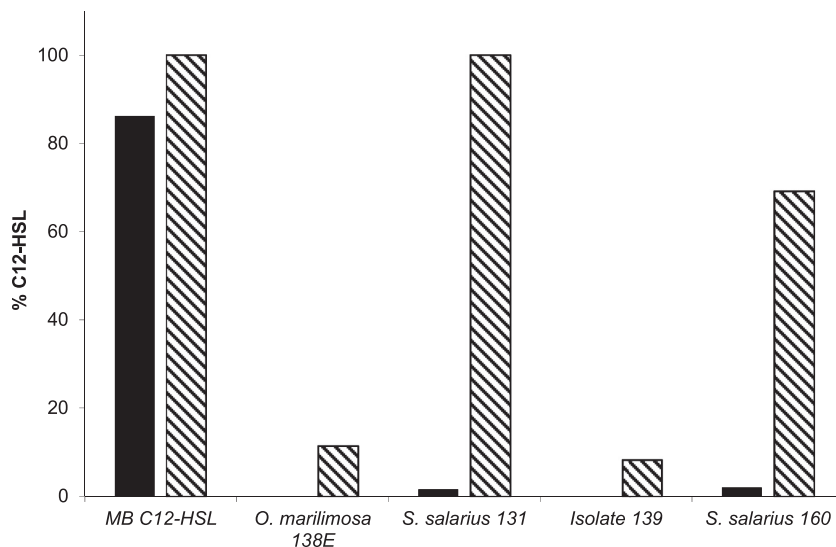


FIG 1 HPLC-MS analysis of degradation of C_{12} -HSL in the culture media of the four selected strains with broad QQ activity with respect to control of marine broth (MB) supplemented with C_{12} -HSL at $50 \mu\text{M}$ (black bars). Spent culture media were acidified to pH 2 in order to allow the recovery of the lactone ring after lactonolysis (hatched bars).

vation of AHLs derived from high pH values in the culture media in these strains.

The acidification of supernatants of the 4 strains capable of degrading C_4 -, C_6 -, C_{10} -, and C_{12} -HSLs allowed the detection of lactonase activity in isolates 131 and 160, as indicated by the recovery of the AHL concentration after acidification (Fig. 1). The 4 strains with wide-spectrum QQ activity were identified by amplification and partial sequencing of the 16S rRNA gene (sequences deposited in GenBank under the accession numbers JQ429320 to JQ429323). All 4 isolates belonged to genera typical of marine environments, and none of them belonged to genera in which isolates had been previously described to have QQ activity (21). Isolates 131 and 160 belonged to the same species: *Salinicola salarius* (5) with similarities with the 16S rRNA gene sequence of 98.4% and 99.9%, respectively. The species closest to isolate 138E is *Olleya marilimosa* (99.9%), while strain 139 would represent a new species close to *Maribacter ulvicola* (94.7%).

QQ genes in metagenomic collections. Due to the limitations of estimates of enzymatic activity based on cultivable bacteria in marine environments (4), a search for putative QQ enzymes was also carried out in marine metagenomes in order to estimate the frequency of these genes. Only metagenomic collections of long reads (>400 bp) were used in this study: the microbial seawater metagenomes of the Global Ocean Sampling (GOS) project (19, 22), samples from the North Pacific subtropical gyre (10-, 70-, 130-, 200-, 500-, 770-, and 4,000-m depths [6, 13]), three samples from whale carcasses (20), and two from an Antarctic marine bacterioplankton community (8). The Waseca County Farm Soil Metagenome (20) was also included for comparison with terrestrial habitats. Environmental metagenomic reads were blasted (BLASTX) against the set of the problem proteins, which included the QQ enzymes lactonase (172 sequences) and acylase (42 sequences) with experimentally proved activity (sequences used in the searches are included in the supplemental material).

The search carried out in the GOS metagenome collection (19, 22) yielded a total of 958 hits: 218 lactonases and 740 acylases.

Among the lactonases, 97% of them presented the β -lactamase or the phosphotriesterase (PTE) domain, characteristic of these QQ enzymes. No clear pattern of distribution was found among the different locations of the GOS metagenome collection (Fig. 2). The normalized relative frequency of these genes, calculated as the number of hits obtained divided by the number of Mbp used, for the whole GOS collection was $0.053 \text{ hits Mbp}^{-1}$, while the same search carried out in the other marine metagenomic collections revealed much higher frequencies, especially in the Antarctic metagenome (8), with a total frequency of $0.435 \text{ hits Mbp}^{-1}$ (Fig. 2). For comparison of sequence abundances, the same search was carried out with different protein sequences related to nutrient acquisition and oxidative metabolism in marine bacteria (15), revealing that the frequency of QQ sequences in these collections is in general higher than the frequency of extremely common oxidative enzymes characteristic of proteobacteria, such as DmdA (15), and even approaches the frequency of the ubiquitous ammonium transporter gene *amt* (Fig. 3). Therefore, as for cultivable bacteria, the frequency of QQ genes in marine metagenomes indicates that AHL degradation is an important process in the sea. The search in a farm soil metagenome revealed a high prevalence of this activity in the soil genomes as well. This is supported by the results of Wang and Leadbetter (23), who observed a rapid AHL degradation in natural samples of soils.

As for cultivable bacteria, the data derived from metagenomic searches should be taken with caution since diverse constraints inherent to this type of search may affect the results. It has been shown for several enzymatic families that even a high level of similarity did not ensure the conservation of the function across a protein family. This was the case for the PTE family to which the lactonase QsdA from *Rhodococcus erythropolis* belongs, as *qsdA* homologues from other bacteria do not allow for the degradation of AHLs (1). On the other hand, in order to minimize the number of false-positive sequences retrieved, a high similarity threshold was set in the search (50% similarity in 70% of the sequence). This high selective criterion may have excluded some of the active se-

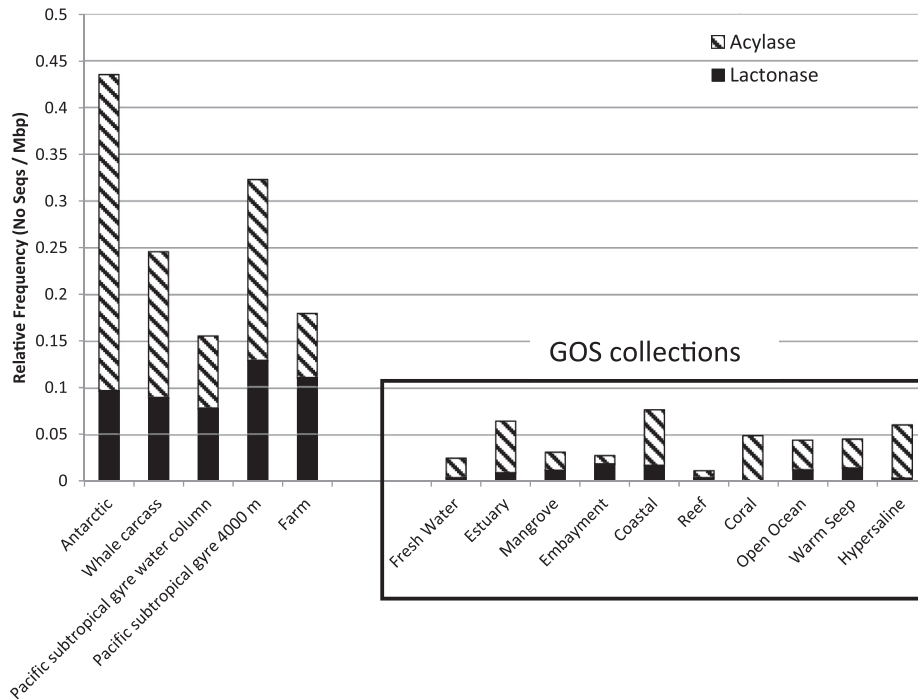


FIG 2 Distribution of sequences containing acylases and lactonases in different marine and farm metagenome collections, including GOS collections classified by different habitats. The number of sequences found was normalized by the number of megabases sequenced in each habitat.

quences, since many known QQ sequences do not fit those similarity boundaries. Nevertheless, and even taking into account the limitations of the methodologies applied, both cultivable bacteria and metagenomic data seem to indicate that the capacity for AHL degradation is not a negligible activity in the sea.

The high abundance of marine cultivable bacteria with QQ activity found and the high frequency of QQ genes in marine metagenomes contrast with the low bacterial population encountered in the open sea and the low chemical stability of AHLs in seawater (3, 11). This discrepancy could indicate that the wide

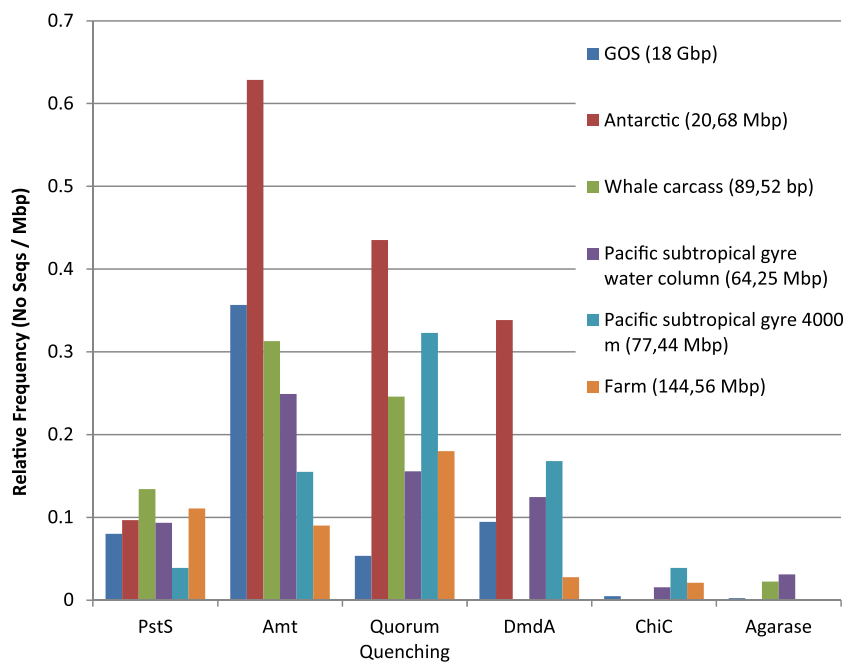


FIG 3 Relative frequencies of QQ sequences present in metagenome collections in comparison with frequencies of other genes involved in nutrient acquisition, such as the phosphate ABC-type transporter (PstS) and the ammonium transporter (Amt), and other genes involved in oxidative metabolism, such as the dimethylsulfoniopropionate demethylase (DmdA), the group I chitinases (ChiC), and agarase.

variety of degradative enzymatic strategies in the sea includes promiscuous enzymes able to use AHL QS signals as an additional energy source, instead of a more dedicated role of AHL degradation enzymes in the interference with bacterial communication processes. The frequency of QQ genes of marine metagenomes, being higher than or comparable to that obtained for genes related to nutrient acquisition and oxidative metabolism in marine bacteria (Fig. 3), seems to support this hypothesis. Moreover, two of the isolates with wide-spectrum degradation activity against AHLs (*O. marilimosa* 138E and strain 139, close to *M. ulvicola*) belonged to the *Bacteroidetes*, a group that harbors a great variety of enzymes related to the mineralization of high-molecular-weight organic matter and constitutes the main degraders of organic matter in the bacterioplankton (2). Therefore, we cannot disregard the possibility that this high AHL degradation activity is not solely related to the interference with QS systems, since some QQ enzymes show homology to enzymes showing other metabolic activities (7, 16), which opens the possibility that the capacity for degradation of AHLs is only one of the functions of these enzymes in the sea. Further studies exploring AHL degradation activities *in situ* by marine samples and the capacity of QQ enzymes to degrade other molecules with structures similar to AHLs would be helpful in clarifying the environmental relevance of QQ activities.

Nucleotide sequence accession numbers. The sequences of the 4 strains with wide-spectrum QQ activity identified by amplification and partial sequencing of the 16S rRNA gene were deposited in GenBank under the accession numbers [JQ429320](#) to [JQ429323](#).

ACKNOWLEDGMENTS

This work was financed by a grant from Consellería de Innovación e Industria, Xunta de Galicia PGDIT06PXIB200045PR, and the foundation Pedro Barrié de la Maza. Manuel Romero was supported by an FPU fellowship from the Spanish Ministry of Science and Education. Anabelen Martín-Cuadrado was supported by a Juan de la Cierva scholarship from the Spanish Ministerio de Ciencia e Innovación.

We thank the Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, for the use of the infrastructure needed to take oceanic samples.

REFERENCES

- Afriat L, Roodveldt C, Manco G, Tawfik DS. 2006. The latent promiscuity of newly identified microbial lactonases is linked to a recently diverged phosphotriesterase. *Biochemistry* 45:13677–13686.
- Bauer M, et al. 2006. Whole genome analysis of the marine *Bacteroidetes* “*Gramella forsetii*” reveals adaptations to the degradation of polymeric organic matter. *Environ. Microbiol.* 8:2201–2213.
- Cicirelli EA, Williamson H, Tait K, Fuqua C. 2008. Acylated homoserine lactone signalling in marine bacterial systems, p 251–272. *In* Winans SC, Bassler BL (ed), *Chemical communication among bacteria*. ASM Press, Washington, DC.
- Cottrell MT, Kirchman DL. 2000. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence *in situ* hybridization. *Appl. Environ. Microbiol.* 66:5116–5122.
- De la Haba RR, Sánchez-Porro C, Márquez MC, Ventosa A. 2010. Taxonomic study of the genus *Salinicola*: transfer of *Halomonas salaria* and *Chromohalobacter salarius* to the genus *Salinicola* as *Salinicola salarius* comb. nov. and *Salinicola halophilus* nom. nov., respectively. *Int. J. Syst. Evol. Microbiol.* 60:963–971.
- DeLong EF, et al. 2006. Community genomics among stratified microbial assemblages in the ocean’s interior. *Science* 311:496–503.
- Dong YH, Wang LH, Zhang LH. 2007. Quorum-quenching microbial infections: mechanisms and implications. *Philos. Trans. R. Soc. B* 362:1201–1211.
- Ghiglione JF, Murray AE. 2012. Pronounced summer to winter differences and higher wintertime richness in coastal Antarctic marine bacterioplankton. *Environ. Microbiol.* 14:617–629.
- Gram L, Grossart HP, Schlingloff A, Kiorboe T. 2002. Possible quorum sensing in marine snow bacteria: production of acylated homoserine lactones by *Roseobacter* strains isolated from marine snow. *Appl. Environ. Microbiol.* 68:4111–4116.
- Hmelo L, Mincer TJ, Van Mooy BAS. 2011. Possible influence of bacterial quorum sensing on the hydrolysis of sinking particulate organic carbon in marine environments. *Environ. Microbiol. Rep.* 3:682–688.
- Hmelo L, Van Mooy BAS. 2009. Kinetic constraints on acylated homoserine lactone-based quorum sensing in marine environments. *Aquat. Microb. Ecol.* 54:127–133.
- Huang YL, Ki JS, Case RJ, Quian PY. 2008. Diversity and acyl-homoserine lactone production among subtidal biofilm-forming bacteria. *Aquat. Microb. Ecol.* 52:185–193.
- Konstantinidis KT, Braff J, Karl DM, DeLong EF. 2009. Comparative metagenomic analysis of a microbial community residing at a depth of 4,000 meters at station ALOHA in the North Pacific subtropical gyre. *Appl. Environ. Microbiol.* 75:5345–5355.
- Mohamed NM, et al. 2008. Diversity of quorum-sensing signal production of Proteobacteria associated with marine sponges. *Environ. Microbiol.* 10:75–86.
- Moran MA. 2008. Genomics and metagenomics of marine prokaryotes, p 91–129. *In* Kirchman DL (ed), *Microbial ecology of the oceans*, 2nd ed. John Wiley and Sons, Hoboken, NJ.
- Park SY, et al. 2005. Identification of extracellular *N*-acylhomoserine lactone acylase from a *Streptomyces* sp. and its application to quorum quenching. *Appl. Environ. Microbiol.* 71:2632–2641.
- Romero M, Diggle SP, Heeb S, Cámara M, Otero A. 2008. Quorum quenching activity in *Anabaena* sp. PCC7120: identification of AiiC, a novel AHL-acylase. *FEMS Microbiol. Lett.* 280:73–80.
- Romero M, Martín-Cuadrado AB, Roca-Rivada A, Cabello AM, Otero A. 2011. Quorum quenching in cultivable bacteria from dense marine coastal microbial communities. *FEMS Microbiol. Ecol.* 75:205–217.
- Rusch DB, et al. 2007. The Sorcerer II global ocean sampling expedition: northwest Atlantic through eastern tropical Pacific. *PLoS Biol.* 5:e77. doi: 10.1371/journal.pbio.0050077.
- Tringe SG, et al. 2005. Comparative metagenomics of microbial communities. *Science* 308:554–557.
- Uroz S, Dessaux Y, Oger P. 2009. Quorum sensing and quorum quenching: the yin and yang of bacterial communication. *Chembiochem* 10:205–216.
- Venter JC, et al. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. *Science* 304:66–74.
- Wang Y-J, Leadbetter JR. 2005. Rapid acyl-homoserine lactone quorum signal biodegradation in diverse soils. *Appl. Environ. Microbiol.* 71:1291–1299.
- Yates EA, et al. 2002. *N*-Acylhomoserine lactones undergo lactonolysis in a pH-, temperature-, and acyl chain length-dependent manner during growth of *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. *Infect. Immun.* 70:5635–5646.