# Collection, isolation, and characterization of potentially quorum-sensing, hydrolytically active bacteria from sinking particles in Clayoquot Sound

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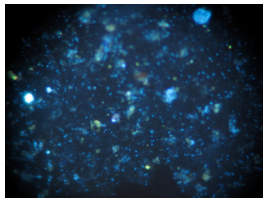
**The degradation of detrital aggregates in the ocean is a major source of dissolved organic carbon and nitrogen and an important contributor to the oceanic carbon cycle. Our study examined the possibility of quorum sensing in bacteria on marine sinking particles with the hypothesis that acyl-homoserine lactone signal molecules regulate hydrolytic enzyme production in certain bacteria. We isolated and identified pure cultivars from particle filtrate from sediment traps in Clayoquot Sound, British Columbia, and tested these for AHL-production. Although no hits were observed using isolates, initial shipboard data indicated the presence of AHLs in trap filtrates, and particle hydrolytic activity increased in response to AHLs. Some isolates exhibit strong chitinase and/or agarase activity, but further characterization is required to determine if this is controlled by quorum sensing.**

## INTRODUCTION

The detritus associated with primary production in the ocean takes the form of sinking particles, which fall through the water column (Simon et al. 2002). These particles are enriched with a unique community of heterotrophic bacteria that produce exoenzymes to degrade particles into dissolved organic matter (Smith et al. 1992). Depending on the depth at which degradation occurs, carbon may be released back to the atmosphere or become solubilized in the deep ocean. Our research focuses on the regulation of exoenzyme production in aggregate-associated bacteria, which we hypothesize to be regulated by cell-cell communication known as quorum sensing.



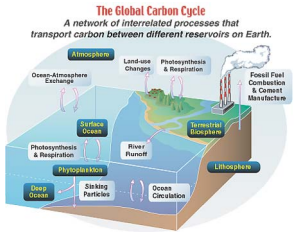



**Figure 1. Fluorescence microscopy of DAPI-stained particles from Clayoquot Sound (left).** Bacteria were at high density in the particulate organic matter we collected as can be seen by the blue prokaryotic pinpoints. **The global carbon cycle includes sinking particles (right).** Dissolved organic carbon may be respired by bacteria and released into the water as $CO_2$. Figure from Paul Preuss, Berkeley National Lab,http://www.lbl.gov/Science-Articles/Archive/sea-carb-bish.html.

We collected sinking particles from the fjords of Clayoquot Sound, British Columbia, Canada in June 2009. Carbon and nitrogen cycling in these fjords has been well studied (Nuwer & Keil 2005), and the site is unique because it contains abundant organic matter of both terrestrial and marine origin, whose contributions fluctuate seasonally. This led us to expect an especially active and diverse microbial community.

We aimed to identify particle-associated bacteria with AHL quorum sensing and to elucidate the genetic components of these systems. We hypothesize that the genes regulated will include those involved in hydrolytic enzyme production, since these enzymes are produced at high concentrations on sinking particles.

### Quorum sensing

Quorum sensing allows bacteria to detect high population density and change gene expression to effect a group behavior (Miller & Bassler 2001). Bacteria secrete and receive quorum sensing signal molecules, like acyl homoserine lactones (AHLs), which increase in concentration with increasing population. At a threshold population density, binding of the AHL signal to its receptor regulates genes that affect the bacterium's behavior. Regulated changes, such as the production of luciferase or exotoxins, are generally advantageous only to large populations of cells (Parsek & Greenberg 1999).

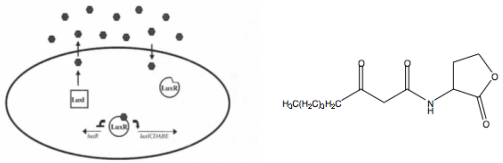
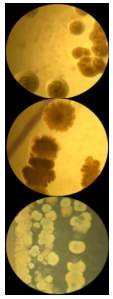


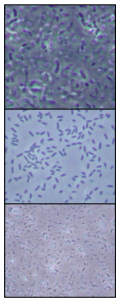




**Figure 2. Quorum sensing in Vibrio fischeri (left).** The AHL signal molecule is produced by LuxI, the autoinducer synthase, and bound by LuxR, the autoinducer receptor, which regulates gene expression when activated. Figure from Miller and Basseler, 2001. **Molecular structure of signal molecule 3-oxo-octanoyl homoserine lactone (right).** AHLs are produced by an autoinducer synthase using S-adenosyl methionine and an acyl-carrier protein (Schaefer et al. 1996).

## METHODS





**Sampling:**
- Gear: Sediment traps, CTD
- Location: Clayoquot Sound fjords (Tranquil Bay and Herbert Inlet)
- Distribution: anoxic and oxic zones
- Timing: 12 or 24 hrs

**Sample processing:**
- On cruise: inoculated 23 original plates with particles from sample
- Streaked mixed bacterial cultures onto a series of new plates in serial dilution
- Described pure colonies on plates
- Made liquid stock cultures of over 100 isolates

**Characterization**
- Microscopy to check for purity, motility and shape
- Biosensor assay for AHL production
- Assay for AHL-induced enzymatic activity
- Identification of bacteria by 16S ribosomal DNA sequence BLAST homologies

## RESULTS

**Table 1. BLAST homologies of 16S rDNA sequences from Clayoquot Sound particle isolates.** Some sequences showed significant homology to multiple GenBank entries, while other were identical to one sequence. GenBank accessed 8/10-8/20/2009.

| Phylogeny | Species | % homology to GenBank sequence | Culture collection number(s) |
|---|---|---|---|
| Flavobacteriales | *Cellulophaga fucicola* | 99% | A295, A300 |
| | *Leeuwenhoekiella aequorea* | 97% | A271, A273 |
| | *Olleya marilimosa* | 100% | A278, A289, A292 |
| Rhodobacterales | *Phaeobacter arcticus* | 100% | A262 |
| | *Pseudorhodobacter incheonensis* | 99-100% | A272, A283 |
| | *Stappia sp. DG* | 98% | A276 |
| Alteromonadales | *Marimonas sp. MWYL1* | 99% | A280 |
| | *Marimonas sp. (pontica)* | 99% | A214, A251, A254 |
| | *Polaribacter dokdonensis* | 95% | A281 |
| | *Pseudoalteromonas S570* | 97% | A222 |
| | *Pseudoalteromonas sp. VPBS2* | 99% | A284, A288, A293 |
| | *Pseudoalteromonas sp. (marina)* | 99% | A208, A209, A212, A213, A217, A218, A220, A221, A233 |
| | *Pseudoalteromonas mariniglutinosa* | 97% | A291 |
| | *Pseudoalteromonas sp. RS-61-B* | 96% | A198 |
| | *Pseudoalteromonas sp. BL* | 97-99% | A227-A230, A232, A234, A236, A239, A241, A258, A260, A263 |
| | *Pseudoalteromonas sp. SC-D1-15* | 98% | A224 |
| | *Pseudoalteromonas sp. BJ19* | 99% | A215 |
| | *Alteromonas sp. BG20* | 97% | A277 |
| Pseudomonadales | *Pseudomonas stutzeri* | 99% | A252 |
| Vibrionaceae | *Vibrio parahaemolyticus, harveyi* | 99% | A255 |
| | *Vibrio sp. (splendidus)* | 97%-99% | A202, A204, A247, A249 |
| | *Vibrio cyclitrophicus* | 99% | A201, A203, A246, A250 |
| | *Vibrio fortis* | 99% | A287 |

- Added 143 new isolates to the Mincer culture collection, with some redundancies
- Identified 61 isolates using 16S rDNA
- Observed chitinase and agarase activities and swarming behaviors indicative of particle-colonization potential and exoenzyme production
- Tested for AHL production under multiple growing environments with no positive identifications

## DISCUSSION

- Particles contained α-proteobacteria, γ-proteobacteria, and bacteriodetes
  - Delong et al. (1993) found γ-proteobacteria and flavobacteria in aggregate-associated communities, but isolated α-proteobacteria only as free-living.
  - Long and Azam (2001) found α-proteobacteria attached to particles in diatom blooms
- Of the cultured isolates sequenced, 57% were *Pseudomonas sp.* and another 14% were *Vibrio sp.*
  - Duplicates due to replicate sampling
  - Could also be due to culturing bias
- Reasons we may not have detected AHL production in our isolates:
  - Quorum sensing by marine bacteria is difficult to express in culture
  - Basic pH affects stability of AHLs (Hmelo and Van Mooy 2009)
  - *Agrobacterium* indicator strain can detect only long-chain AHLs (6-carbon or longer), and its detection of these may actually be inhibited by the presence of short-chain AHLs.

Other approaches currently underway include a metagenomic survey of environmental DNA associated with sinking particles, and organic AHL extraction directly from particle samples.

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