

# Evidence of prokaryotic particle-attached way of life in the dark Atlantic Ocean

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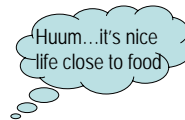
## Is there evidence of a preferential particle-associated life mode of deep-ocean prokaryotes?

Aristegui et al. (2005) and Reinthaler et al. (2006) reported an increasing nucleic acid content per cell with depth indicative for a larger genome size, which, in turn, might point to an **opportunistic life style** of **deep-sea prokaryotes** (Lauro and Bartlett 2008)

QuickTime™ and a  
TIFF (Uncompressed) decompressor  
are needed to see this picture.

## Is there evidence of a preferential particle-associated life mode of deep-ocean prokaryotes?

DeLong et al. (2006) found that **deep-water prokaryotes** are enriched in pilus, polysaccharide and antibiotic synthesis genes, all suggesting a predominately **particle-attached life mode**



QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

## Is there evidence of a preferential particle-associated life mode of deep-ocean prokaryotes?

Ivars-Martinez et al. (2008) described the genome of two different ecotypes (original from "**surface**" and "**deep**" Mediterranean waters) of a common marine heterotrophic gamma-proteobacterium (*Alteromonas macleodii*)

Organic aggregates of relatively **short lifespan** would be more successfully colonized by the "**surface**" ecotype, and **long-lived** particles would favor the "**deep**" one.

## Is there evidence of a preferential particle-associated life mode of deep-ocean prokaryotes?

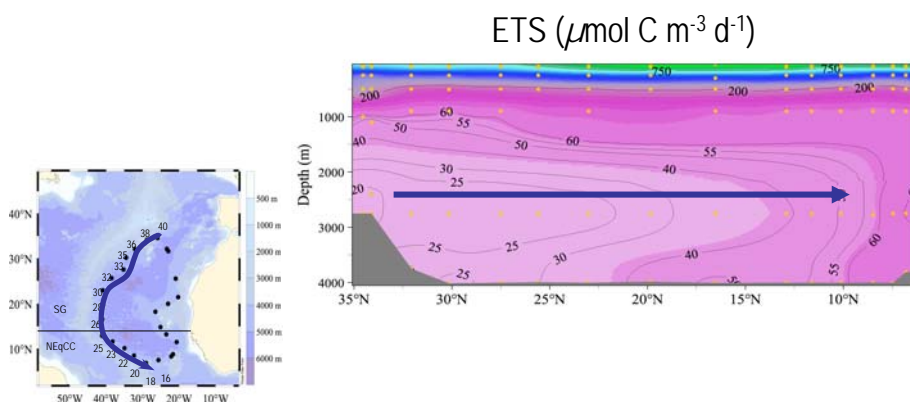
González et al. (2008) reported a substantial number of genes for **attachment** to surfaces or particles, gliding motility, and polymer degradation in the genome of another common bacterioplankton group (*Polaribacter* sp. MED 152 (Flavobacteria)).

In the same PNAS issue, Kirchman (2008) commented the results obtained by González et al. (2008), and suggested that: **“due to the fact that detrital particles are not very numerous in the oceans, the “desert” between particles may be studded with colloids, gels, and various forms of HMW DOM, all potential sources of carbon and energy”**

### Our evidence:

#### a) suspended POM related to prokaryotic metabolism

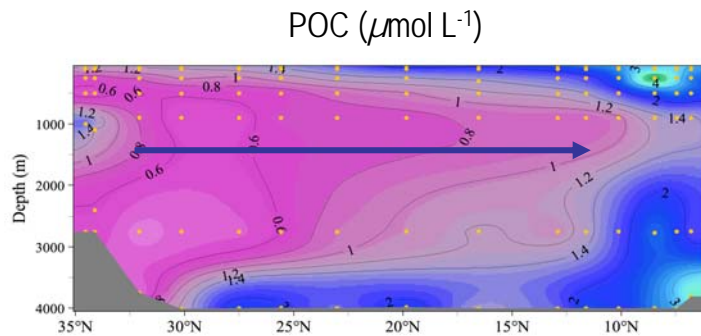
- On a section following the Mid-Atlantic Ridge from 35°N to 5°N, **respiration (ETS)** below 1000 m depth **increased** southwards up to three-fold



Our evidence:

a) suspended POM related to Prokaryotic metabolism

- This latitudinal gradient in the deep-waters was paralleled by a six-fold increase in **suspended particulate organic carbon (POC)** obtained with oceanographic bottles, whereas no trend was apparent in the dissolved organic matter (DOM) distribution



Our evidence:

a) suspended POM related to prokaryotic metabolism

- Significant correlations** between **POM** and **ETS** were obtained in the water masses between 1000 m and 3000 m depth (Antarctic Intermediate Water and the North East Atlantic Deep Water cores)

ETS is **strongly positively correlated** with POC (Spearman's  $\rho = 0.70$ ,  $p < 0.02$ ) in the NEADW, and in the AAIW (Spearman's  $\rho = 0.82$ ,  $p < 0.0001$  for POC and  $0.93$ ,  $p < 0.0001$  for PON)



The strong correlation between POM (collected with oceanographic bottles, considered to be mostly suspended detrital material) and ETS activity supports the hypothesis that deep-water prokaryotic activity depends to a large extent on suspended particles!

## Our evidence:

### b) “Deep” ocean imbalance between POC availability and PCD

Water mass	Depth range (m)	PHP NEqCC	PHP SG	R (ETS) NEqCC	R (ETS) SG
LDW	3500-5000	0.0318 (0.007)	0.0394 (0.011)	6.50 (1.15)	3.09 (0.62)
NEADW	2750	0.0704 (0.027)	0.026 (0.005)	4.42 (0.57)	2.16 (0.25)
AAIW/MSOW	900-1000	0.1724 (0.047)	0.0869 (0.018)	5.90 (0.92)	2.49 (0.38)
OML	250-500	1.4748 (0.47)	1.2169 (0.30)	16.30 (2.07)	13.50 (1.62)
subsurface	100	7.1599 (1.62)	14.302 (2.11)	46.44 (4.86)	46.94 (8.1)
SML	50			129.36 (12)	90.88 (13)

All units in  $\mu\text{mol C m}^{-3} \text{d}^{-1}$ . SE in brackets

- The prokaryotic carbon demand (PCD) was derived from ETS and PHP measurements (**PCD(ETS)**) assuming an R:ETS of 0.09

## Our evidence:

### b) “Deep” ocean imbalance between POC availability and PCD

Water mass	Depth range (m)	PHP NEqCC	PHP SG	R (ETS) NEqCC	R (ETS) SG	PCD (ETS) NEqCC	PCD (ETS) SG
LDW	3500-5000	0.0318 (0.007)	0.0394 (0.011)	6.50 (1.15)	3.09 (0.62)	<b>6.53</b> (1.17)	<b>3.13</b> (0.64)
NEADW	2750	0.0704 (0.027)	0.026 (0.005)	4.42 (0.57)	2.16 (0.25)	<b>4.49</b> (0.60)	<b>2.19</b> (0.26)
AAIW/MSOW	900-1000	0.1724 (0.047)	0.0869 (0.018)	5.90 (0.92)	2.49 (0.38)	<b>6.07</b> (0.98)	<b>2.58</b> (0.40)
OML	250-500	1.4748 (0.47)	1.2169 (0.30)	16.30 (2.07)	13.50 (1.62)	<b>17.77</b> (2.49)	<b>14.72</b> (1.91)
subsurface	100	7.1599 (1.62)	14.302 (2.11)	46.44 (4.86)	46.94 (8.1)	<b>53.60</b> (6.48)	<b>61.24</b> (10.2)
SML	50			129.36 (12)	90.88 (13)		

All units in  $\mu\text{mol C m}^{-3} \text{d}^{-1}$ . SE in brackets

- To further constrain PCD, a prokaryotic growth efficiency (PGE) of 0.04 was used as well (**PCD (PGE)**) (no R data needed)
- Also the **OUR (oxygen utilization rates)** for the mesopelagic North and South Atlantic are given (Jenkins 1982, Jenkins & Wallace 1992, Brea 2008)

Our evidence:

b) “Deep” ocean imbalance between POC availability and PCD

Water mass	Depth range (m)	PCD (ETS) NEqCC	PCD (ETS) SG	PCD (PGE) NEqCC	PCD (PGE) SG	OUR NATl	OUR SATl
LDW	3500-5000	6.53 (1.17)	3.13 (0.64)	0.80 (0.17)	0.99 (0.27)		
NEADW	2750	4.49 (0.60)	2.19 (0.26)	1.76 (0.67)	0.65 (0.12)		
AAIW/MSOW	900-1000	6.07 (0.98)	2.58 (0.40)	4.31 (1.17)	2.17 (0.44)	6.7	11.8
OML	250-500	17.77 (2.49)	14.72 (1.91)	36.87 (11.9)	30.43 (7.3)	14.7	18.3
subsurface	100	53.60 (6.48)	61.24 (10.2)	179.0 (40)	357.5 (52.8)		
SML	50						

- Our **ETS-derived R** rates are in good agreement with the **biogeochemical OUR** estimates
- Available sinking POC (POC Av) was inferred from the POC flux model of Antia et al. (2001) derived from sediment trap records corrected with <sup>230</sup>Th, assuming the range of surface primary production (PP) proposed by these authors for the North Atlantic (12 and 90 mmol C m<sup>-2</sup> d<sup>-1</sup>)

Our evidence:

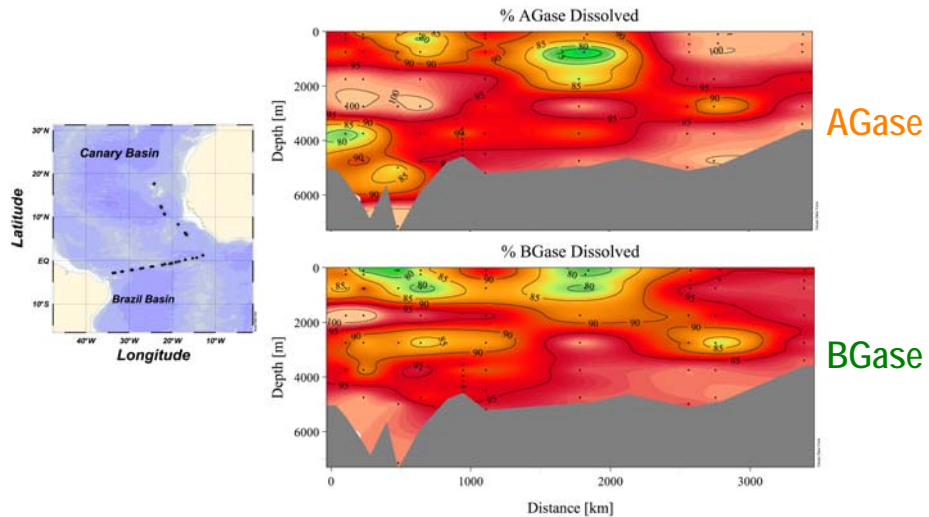
b) “Deep” ocean imbalance between POC availability and PCD

Water mass	Depth range (m)	PCD (ETS) NEqCC	PCD (ETS) SG	PCD (PGE) NEqCC	PCD (PGE) SG	OUR NATl	OUR SATl	POC Av PP=90	POC Av PP=12
LDW	3500-5000	6.53 (1.17)	3.13 (0.64)	0.80 (0.17)	0.99 (0.27)			0.18 (0.04)	0.01 (0.001)
NEADW	2750	4.49 (0.60)	2.19 (0.26)	1.76 (0.67)	0.65 (0.12)			0.33 (0.06)	0.01 (0.002)
AAIW/MSOW	900-1000	6.07 (0.98)	2.58 (0.40)	4.31 (1.17)	2.17 (0.44)	6.7	11.8	2.07 (0.41)	0.06 (0.012)
OML	250-500	17.77 (2.49)	14.72 (1.91)	36.87 (11.9)	30.43 (7.3)	14.7	18.3	12.12 (1.75)	0.34 (0.05)
subsurface	100	53.60 (6.48)	61.24 (10.2)	179.0 (40)	357.5 (52.8)			84.54 (16.9)	2.39 (0.478)
SML	50							265.1 (56.5)	7.49 (1.59)

These results indicate that microbial life in the dark ocean is likely more dependent on slowly sinking or buoyant, laterally advected suspended particles than hitherto assumed

Our evidence:

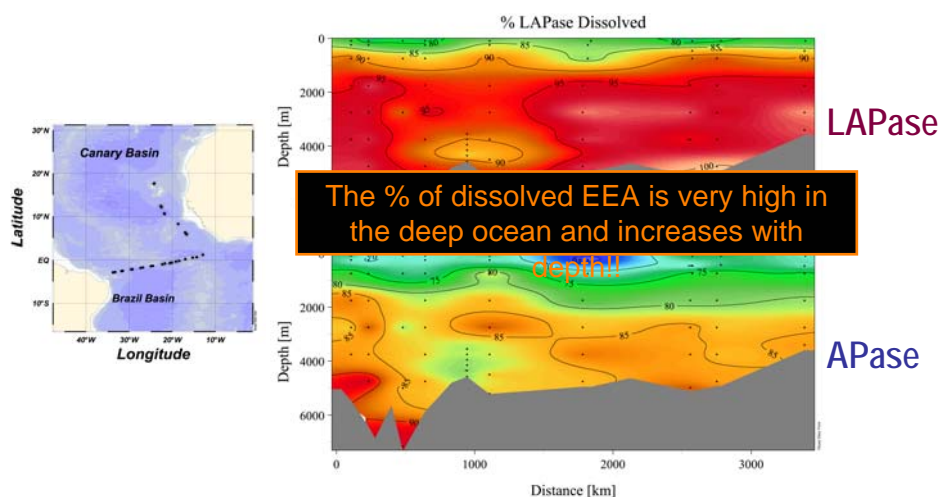
c) EEA is in dissolved fraction in the dark ocean



- Contribution of dissolved (passing through 0.2  $\mu\text{m}$ ) to total extracellular enzymatic activity (EEA) for alpha- and beta-glucosidase (AGase, BGase)

Our evidence:

c) EEA is in dissolved fraction in the dark ocean

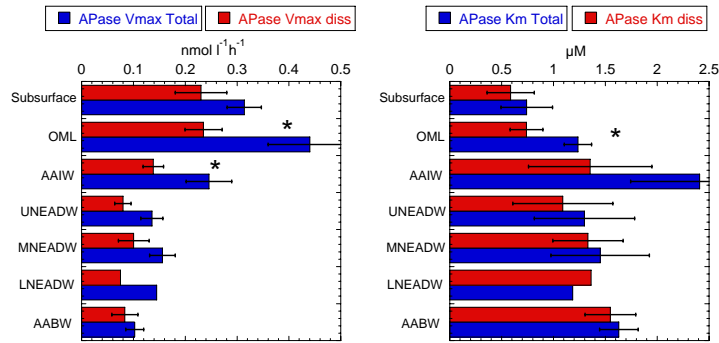


- Contribution of dissolved to total EEA for leucine aminopeptidase (LAPase) and alkaline phosphatase (APase)

Our evidence:

c) EEA is in dissolved fraction in the dark ocean

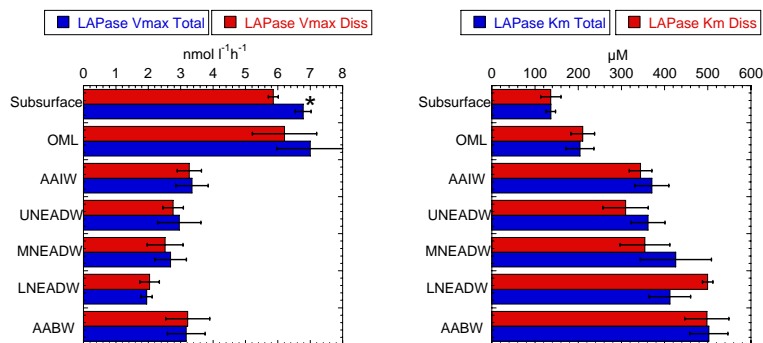
- Profiles of **APase and LAPase kinetics** for the total and dissolved fractions were also obtained



- Similar pattern in **Dissolved** and **Total** APase (APase V<sub>max</sub> decreases and Km increases with depth for both), only significant differences (\*t-test; p < 0.05) in few shallower water masses

Our evidence:

c) EEA is in dissolved fraction in the dark ocean



The kinetic parameters of dissolved and total LAPase and of APase were similar throughout the water column, suggesting that the dissolved and particulate EEA are of the same origin

Our evidence:

c) EEA is in dissolved fraction in the dark ocean

Spearman's  $\rho$ ; \* $p < 0.05$ , \*\* $p < 0.01$

	PHP	POC	PON	POC : PON
Total AGase	0.45**	0.49**	0.15	-0.29*
Total BGase	0.48**	0.45**	0.14	-0.29*
Total LAPase	<b>0.52**</b>	<b>0.51**</b>	0.20	-0.30**
Total APase	<b>0.67**</b>	<b>0.64**</b>	0.36**	-0.31**
Dissolved AGase	0.52**	0.51**	0.18	-0.27**

The significant relation found between total and dissolved EEA, and between EEA and the POM pool and the prokaryotic metabolism, suggests an **active response** to the organic matter pool by the prokaryotes

	PHP	POC	PON	POC : PON
% LAPase diss.	<b>-0.67**</b>	<b>-0.48**</b>	-0.35*	0.19
% APase diss.	<b>-0.62**</b>	<b>-0.36**</b>	-0.24	0.18

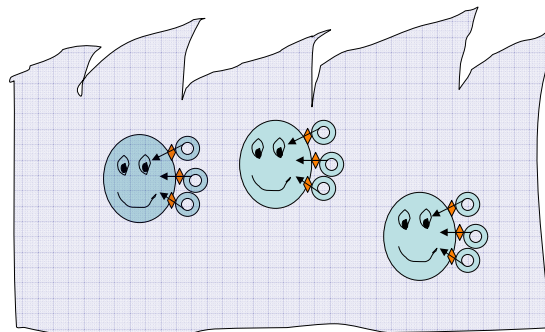
- Significant correlations were found between the free and total EEA with prokaryotic production and the POM pool, particularly LAPase and APase

Our evidence:

c) EEA is in dissolved fraction in the dark ocean

Is this finding surprising?

- Yes, as it is commonly assumed that free-living prokaryotes exhibit a tight hydrolysis-uptake coupling with the **ectoenzymes** attached to the cell wall or in the periplasmic space where cleavage of **macromolecular substrate** occurs in intimate vicinity to the **uptake sites** of cleavage products (Hoppe et al. 2002)

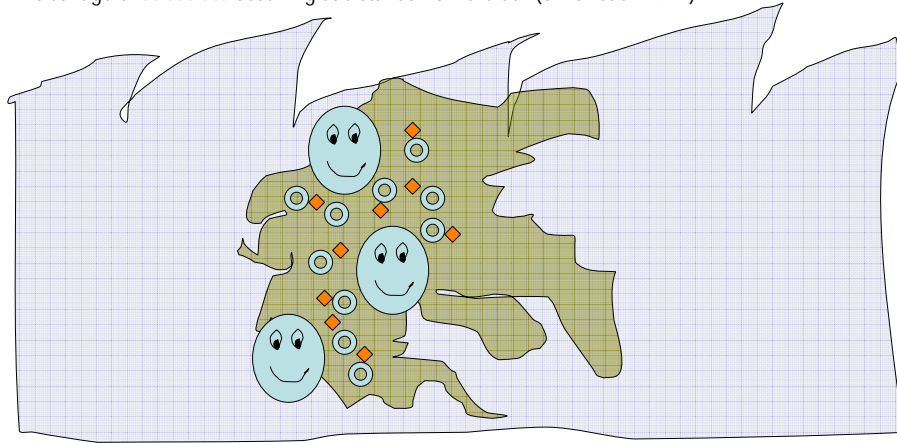


Our evidence:

c) EEA is in dissolved fraction in the dark ocean

Is this finding surprising?

- In contrast, **particle-associated prokaryotes** have been reported to exhibit a loose hydrolysis-uptake coupling with the **extracellular enzymes released** into the particle and enzymatic cleavage of **substrate** occurring at distance from the cell (Smith et al. 1992)



Our evidence:

c) EEA is in dissolved fraction in the dark ocean

...Moreover....

- Vetter et al. (1998) **modelling** and Vetter and Deming (1999) **empirically** report that **released bacterial EE** can produce **sufficient hydrolysate from POC** to support growth in the absence of any other significant source of DOC and **without direct contact** between cell and particulate substrate (if the substrates are within a well-defined distance of the enzyme source)
- The release of EE by the **particle-associated prokaryotes** into the particle might help maintaining relatively high metabolic activity as compared to their **free-living** counterparts. In fact, **bacterial "foraging" theory** suggests that independent of possible assemblage differences, individual bacteria may produce more EE in **diffusionally constrained areas** such as **particle aggregates**, where return of hydrolysate is potentially high (Vetter et al., 1998)
- Allison (2005) has argued that microbes releasing EE have a competitive advantage over other microbes in environments with low rates of enzyme diffusion, such as sediments or **particles**

## Conclusions & Implications

- Several **evidences** were found in the dark Atlantic Ocean:
  - Correlation between the **particulate fraction of the OM pool** and the **activity** of prokaryotes
  - Imbalance between the **PCD** and estimates of **C availability** derived from sinking fluxes
  - High percentage of **free EEA** and a tendency to increase **with depth**
- The notion that **microbes grow associated with (colloidal) particles in the deep ocean**, where they may maintain high metabolic activities, explains the discrepancy found between the PCD and the sinking POC flux and higher release of EEs

## Conclusions & Implications

- Our data suggest that **microbial activity in the dark ocean might be more bound to particulate and colloidal material than hitherto assumed**
- However, the collection of samples in the water column with standard methods frequently **leads to the disruption of the size-continuum** of organic matter, possibly altering the colloidal micro-environment where microbial assemblages develop
- Thus, **new sampling strategies are needed** to selectively collect deep ocean particles to decipher the actual role of particle-attached versus free-living microbes in the deep ocean's biogeochemical cycles

Thank you very much for  
your attention!!

