Metabolic adaptations to marine environments: molecular diversity and evolution of ovothiol biosynthesis in Bacteria

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running title: molecular diversity of ovothiol biosynthesis in bacteria

Supplementary Table: *In silico* identification of cytochrome C oxidase subunit I in the genomes of bacteria annotated as anaerobic.

Species	Environment	Oxygen tolerance	Cytochrome C oxidase sub I	% Identity	E-value
Desulfobulbus sp. Tol-SR	terrestrial	anaerobic	KGO34979.1	39.43	2,00E-119
Thauera sp. 27	freshwater	anaerobic	WP_002940817.1	51.02	0.0
Halomonas sp. GFAJ-1	marine	anaerobic	WP_009097632.1	52.05	0.0
Geobacter uraniireducens	marine	anaerobic	WP_011937350.1	43.04	2,00E-140
Geobacter bemidjiensis	terrestrial	anaerobic	WP_012528484.1	40.29	4,00E-136
Geobacter sp. M21	marine	anaerobic	WP_012773757.1	39.54	5,00E-124
Paludibacter propionicigenes	terrestrial	anaerobic	WP_013446430.1	41.21	3,00E-142
Geobacter sp. M18	terrestrial	anaerobic	WP_015718401.1	41.24	2,00E-135
Proteiniphilum acetatigenes	marine	strictly anaerobic	NI		
Sulfurimonas hongkongensis	marine	strictly anaerobic	NI		
Halomonas boliviensis	multiple	facultative anaerobic	WP_007114357.1	52.29	0.0

* NI= not identified

Supplementary Figure 1

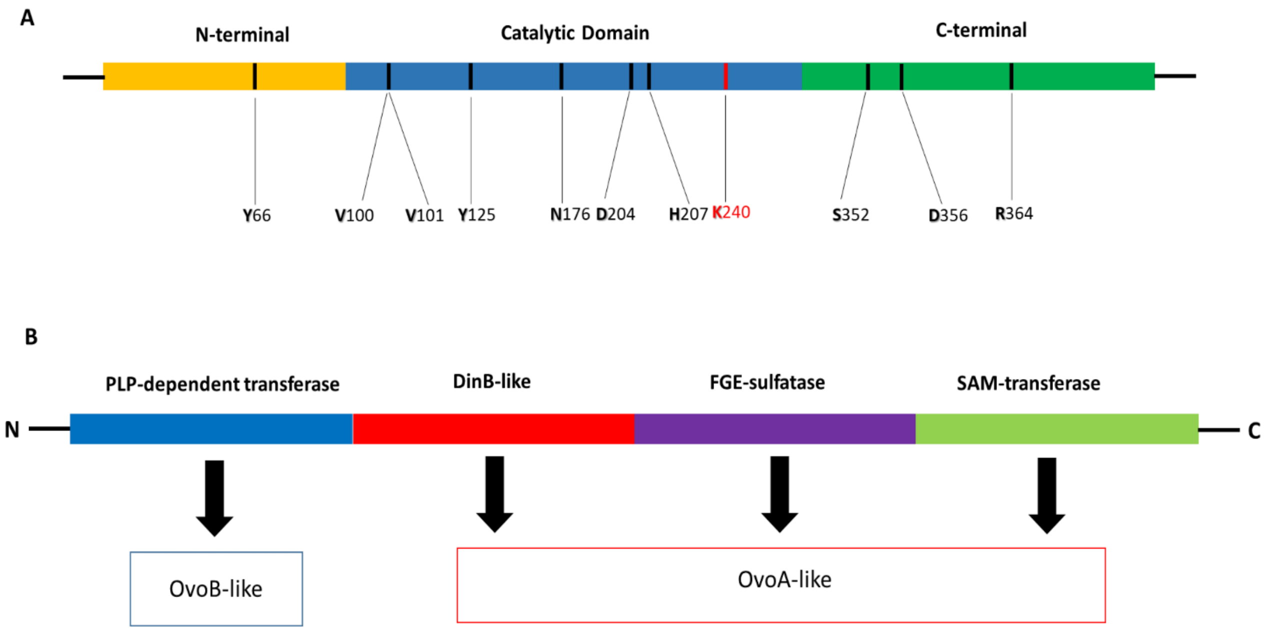
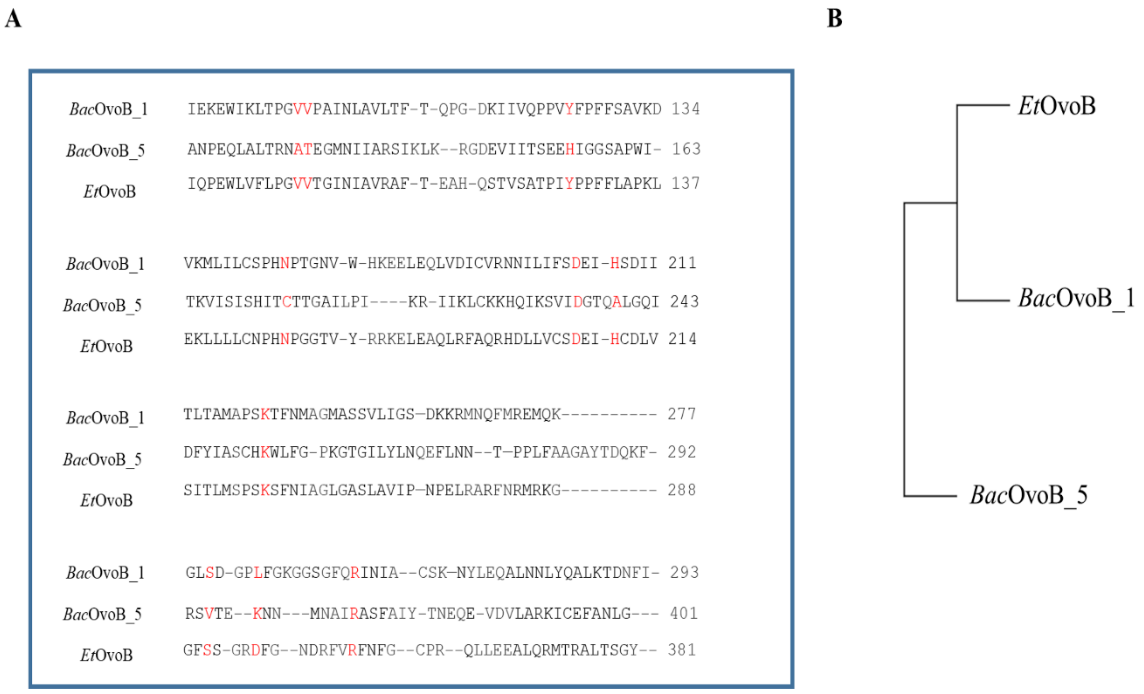


Fig. S1. (A). Schematic representation of bacterial OvoB primary structure. The N-terminal portion is highlighted in orange, the catalytic domain in blue, and the C-terminal portion in green. The residues involved in the enzymatic lyase activity and conserved in bacterial OvoB sequences are indicated by arrows, the numeration refers to *Et*OvoB. (B) Schematic representation of OvoB-OvoA fused protein in Hydrozoa. The PLP-dependent transferase domain, responsible for OvoB-like activity, is highlighted in blue, the DinB domain in red, the FGE-sulfatase domain in violet and the SAM-transferase domain in green. These three last domains are responsible for OvoA-like activities.

Supplementary Figure 2.



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BacOvoB_1   IEKEWIKLTPGVVPAINLAVLTF-T-QPG-DKIIVQPPVYFPFFSAVKD 134
BacOvoB_5   ANPEQLALTRNATEGMNIIARSIKLK--RGDEVIITSEEHIGGSAPWI- 163
EtOvoB      IQPEWLVFLPGVVTGINIAVRAF-T-EAH-QSTVSATPIYPPFFLAPKL 137

BacOvoB_1   VKMLILCSPHNPTGNV-W-HKEELEQLVDICVRNNILIFSDEI-HSDII 211
BacOvoB_5   TKVISISHITCTTGAILPI----KR-IIKLCKKHQIKSVIDGTQALGQI 243
EtOvoB      EKLLLLCNPHNPGGTV-Y-RRKELEAQLRFAQRHDLLVCSDEI-HCDLV 214

BacOvoB_1   TLTAMAPSKTFNMAGMASSVLIGS—DKKRMNQFMREMQK---------- 277
BacOvoB_5   DFYIASCHKWLFG-PKGTGILYLNQEFLNN--T—PPLFAAGAYTDQKF- 292
EtOvoB      SITLMSPSKSFNIAGLGASLAVIP—NPELRARFNRMRKG---------- 288

BacOvoB_1   GLSD-GPLFGKGGSGFQRINIA--CSK—NYLEQALNNLYQALKTDNFI- 293
BacOvoB_5   RSVTE--KNN---MNAIRASFAIY-TNEQE-VDVLARKICEFANLG--- 401
EtOvoB      GFSS-GRDFG--NDRFVRFNFG--CPR--QLLEEALQRMTRALTSGY-- 381
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Fig. S2 (A) Multiple alignment between *Et*OvoB and *Bacteroidetes* OvoB-like protein. Sequence alignment of *Et*OvoB, putative C-S lyase protein (NBC82978.1; *Bac*OvoB_1) and aminotransferase class V-fold PLP-dependent enzyme (NBV57240.1; *Bac*OvoB_5). The residues necessary for the enzymatic activity of OvoB are highlighted in red. We observed high conservation of the residues involved in OvoB-like activity, especially for *Bac*OvoB_1. (B) Structure-based phylogenetic relationship between *Et*OvoB, *Bac*OvoB_1 and *Bac*OvoB_5.

Supplementary Figure 3.

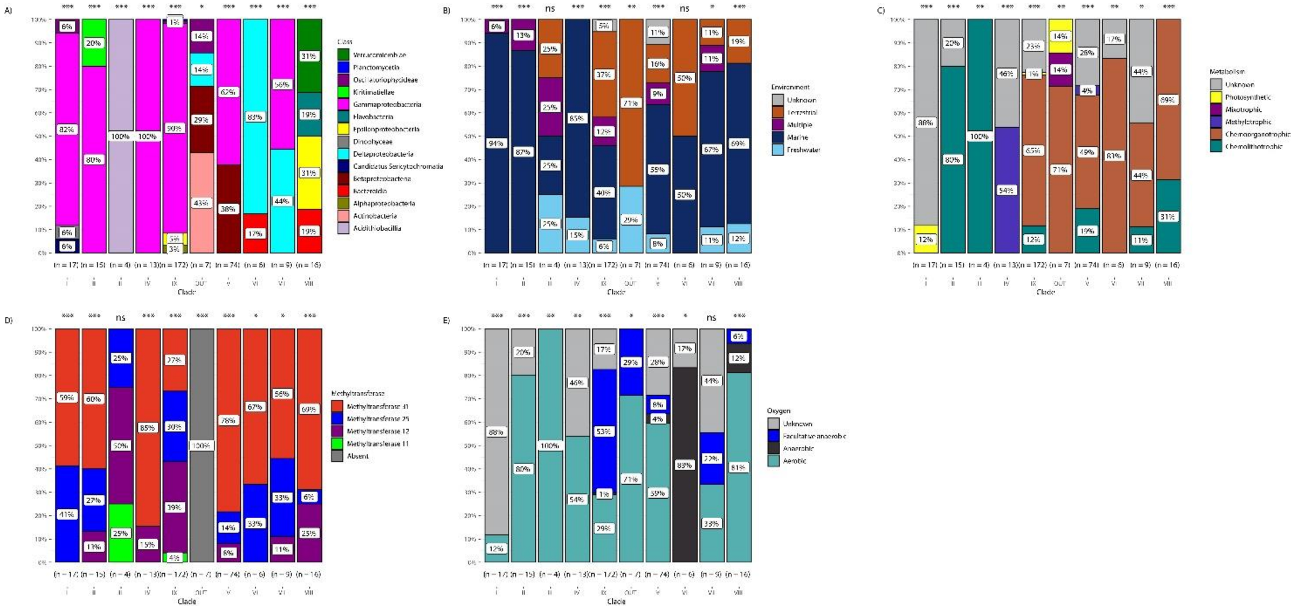

Fig. S3. Differences in taxonomic distribution and metabolic traits among clades of OvoA phylogenetic tree. Partitioning of A) prokaryotic classes, B) isolation environment, C) metabolism, D) MT domain and E) oxygen requirement among the 9 clades identified for OvoA phylogenetic tree. For each plot, goodness-of-fit and adjusted p-values are reported.

Supplementary Figure 4.

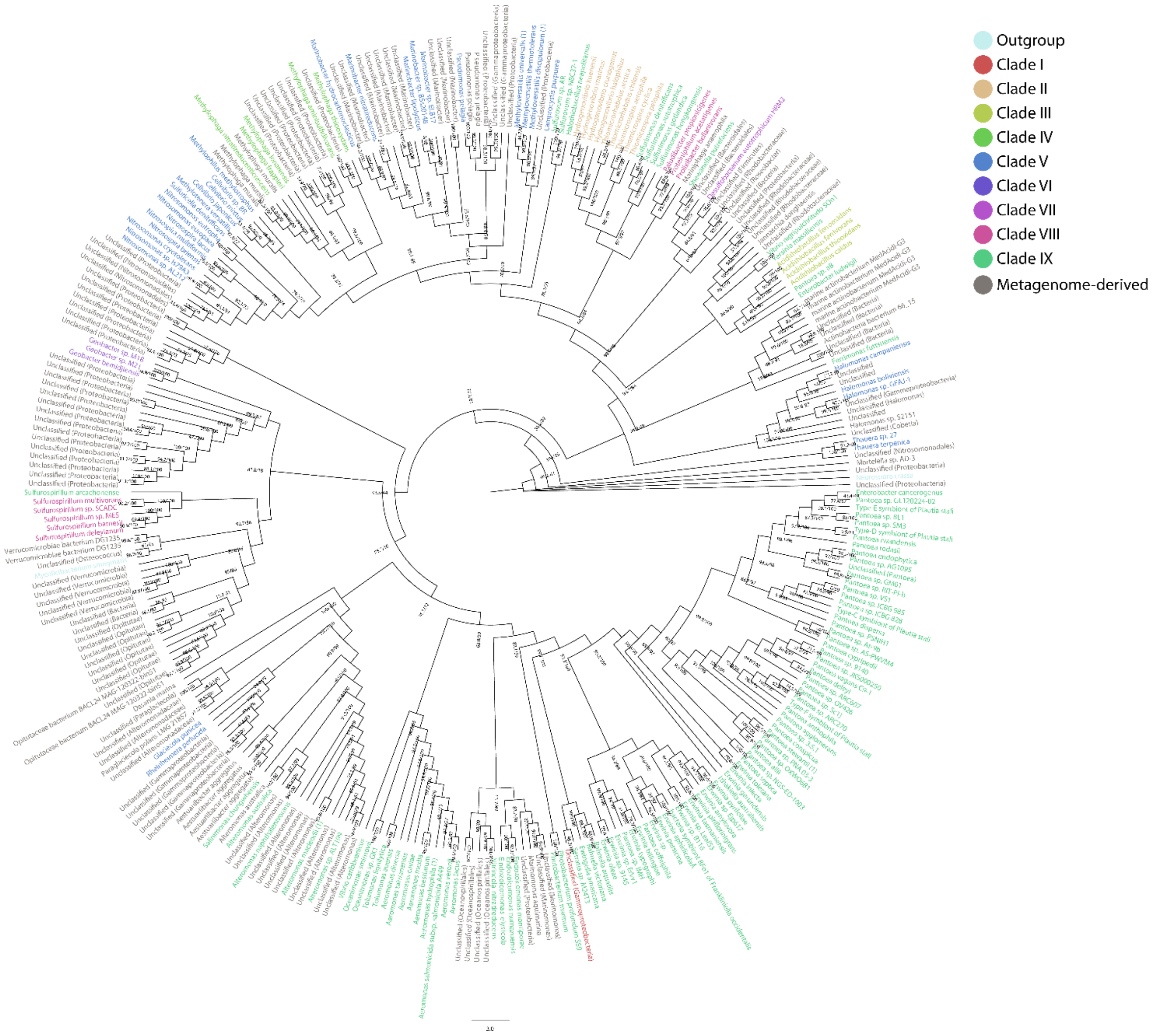

Fig. S4. Phylogenetic tree of OvoB-like sequences in bacteria. The phylogenetic tree resulting from multiple sequence alignment of OvoB-like sequences from both the NCBI database and the Ocean Gene Atlas webserver was visualized using FigTree and colored according to the clades previously identified for the OvoA-like proteins. Labels on branching nodes report both ultrafast bootstrap (UFboot) and the SH-aLRT test results.