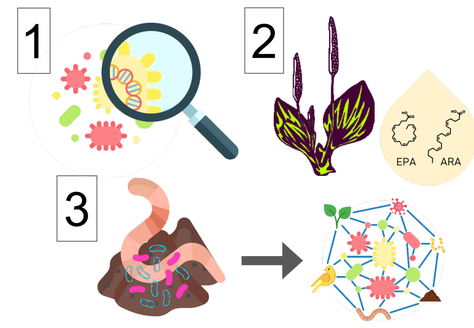
# Searching for novel polyunsaturated fatty acid producers in terrestrial ecosystems implicated in rare-lipid provisioning and ecological services

Stephanie L. Schnorr[1], Daniela Leuzzi[2,3], David Berry[1]

Division of Microbial Ecology, Center for Microbiology and Environmental Systems Science, University of Vienna | Unit of Microbiome Science and Biotechnology, Department of Pharmacy and Biotechnology (FaBit), Alma Mater Studiorum – University of Bologna | Fano Marine center, the Inter-institute Center for Research on Marine Biodiversity, Resources and Biotechnology, Fano Italy

Newly elucidated anaerobic synthase for long-chain polyunsaturated fatty acids (PUFAs) prompts new concepts about nutritional acquisition of prokaryote PUFAs by host metazoan organisms.

PUFAs are essential components of cellular membranes and are precursors for important cell-signaling molecules such as eicosanoids for all metazoans. **Bacterial production using a type I FAS/PKS system was resolved in 2001**. This polyunsaturated fatty acid gene (***pfa***) may provide **wide-scale PUFA production by prokaryotes** and has intrigued namely **two scientific enterprises**: **1)** understanding the ecological spread of synthase competence to find novel producer organisms, and **2)** leveraging the PKS-like system for heterologous expression towards commercial mass production. This study adds a third enterprise **using *Shewanella* bacteria isolated from the earthworm intestinal tract** to **3)** investigate a novel hypothesis that bacterial producers of PUFAs and other structural lipid alkyl chains may use them to alter the fluidity of the lipid membrane, both cross-sectionally and laterally and plays an adaptive role for dissimilatory metal reduction.




## Methods: 2-phased approach

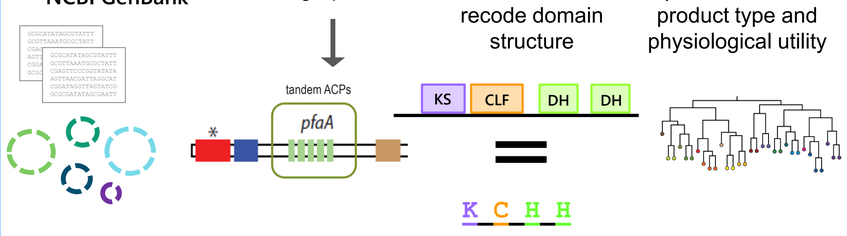
### Informatics workflow: Find synthases among prokaryotes

**1** Download complete whole genomes **NCBI GenBank**

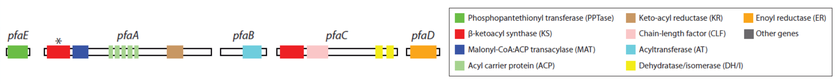
**2** Search for PUFA synthase using ACP fingerprint

**3** Annotate full synthase complex with HMMER and recode domain structure

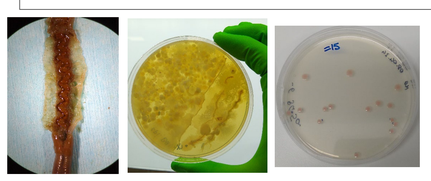
**4** Hierarchical clustering of domain structural sequence to estimate product type and physiological utility



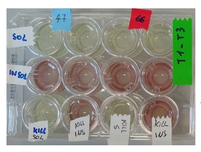

**PUFA synthase multi-domain architecture among 5 *pfa* genes** (from ref. 10)



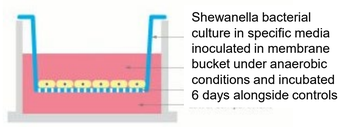

### Lab: *Shewanella* iron reduction & PUFA synthase expression



**1** *Shewanella* isolates from gut contents of wild *Aporrectodea* and *Lumbricidae* earthworms are PUFA-producer candidates – identified from ***pfa*** gene. *Shewanella* are established dissimilatory metal reducers using multiple extracellular electron transport (EET) mechanisms, perhaps coupled to the need for fluidizing membrane lipids.



Soluble and insoluble ferric iron ($Fe^3$) in lower compartment



Shewanella bacterial culture in specific media inoculated in membrane bucket under anaerobic conditions and incubated 6 days alongside controls

**2** Perform incubation with soluble and insoluble ferric iron ($Fe^3$) and measure reduction activity and concurrent PfaA-KS gene expression to understand if PUFA production is linked to membrane performance during long-range EET

## Results

### Informatically find novel organisms w/ PUFA synthase

**GenBank genomes queried for PUFA synthase**

| Lineage | Initial total | Synthase positive | Synthase annotated |
|---|---|---|---|
| Archaea | 5838 | 149 | 24 |
| Bacteria* (complete genomes) | 29447 | 257 | 238 |
| Fungi | 3385 | 91 | 0 |
| Invertebrate | 450 | 0 | 0 |
| Plant | 341 | 0 | 0 |
| Protozoa | 416 | 4 | 0 |
| | **39,877** | **501** | **262** |

Occurrence rate overall = 1.26%

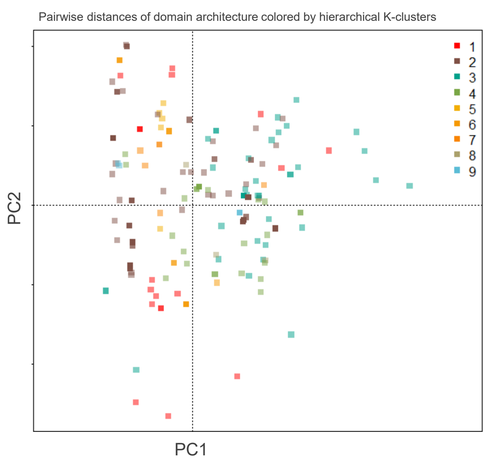
Bacteria = 0.87%

Unknown Archaean and Fungal competences

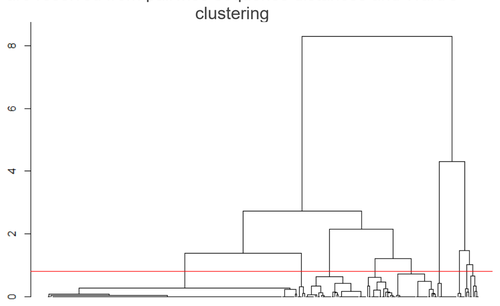
Complete genome annotated protein sequences were downloaded and queried using HMMER with the universal phosphopantetheine binding sites of the acyl-carrier protein. Hits were curated to select those presenting the distinct tandem-repeat fingerprint of the ACP domains in the ***pfa*** synthase complex (approximately 20k base pairs)

**GenBank complete genome datasets mined for *pfa* synthase complex and domains annotated and recoded to create structural sequence information**

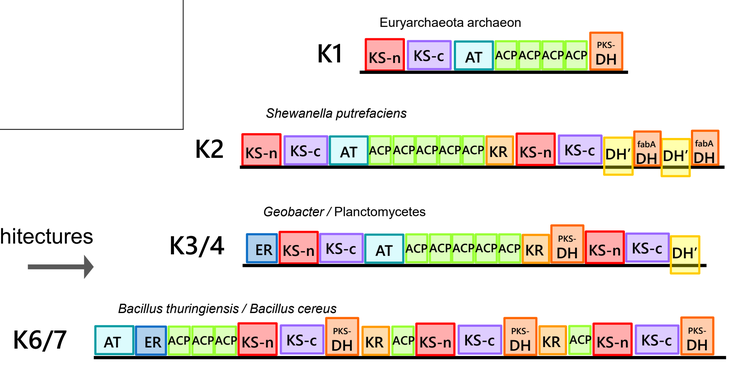
Domain architecture shows stratified clusters along PC1 among the annotated prokaryote genomes, with 4 interspersed clusters dominating the representation.




9 distinct cluster groups from the 273 ***pfa*** cluster architectures are resolved from pairwise sequence distances and Ward's clustering

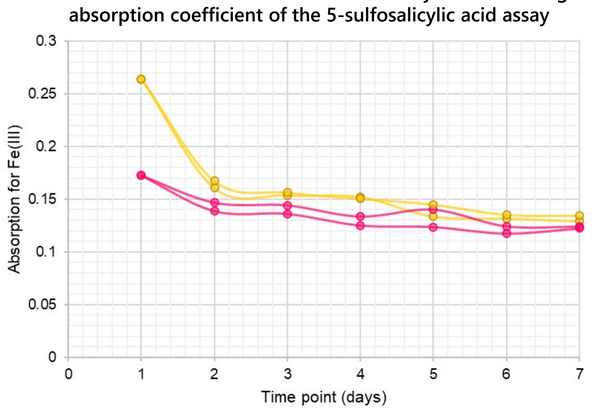


Representative domain architectures of the 4 dominant clusters




### Extracellular iron reduction and PUFA synthase expression assays from time-series

Ferric iron reduction over time indicated by the decreasing absorption coefficient of the 5-sulfosalicylic acid assay



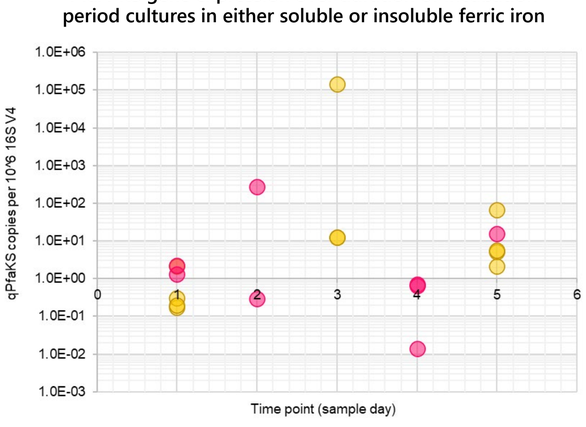

Ferric iron reduction proceeded for both soluble and insoluble media solutions at roughly similar rates over duration of incubation period, indicating that cultures were using reduction mechanisms with similar efficiency

Soluble Fe(III)

Insoluble Fe(III)

However, despite similar rates of ferric reduction, cultures with solubilized ferric iron generally expressed higher PfaA-KS RNA copies relative to 16S rRNA copies over sampling time. This means the cells may be in conditions permitting higher membrane fluidity

PfaA-KS gene expression over duration of incubation period cultures in either soluble or insoluble ferric iron



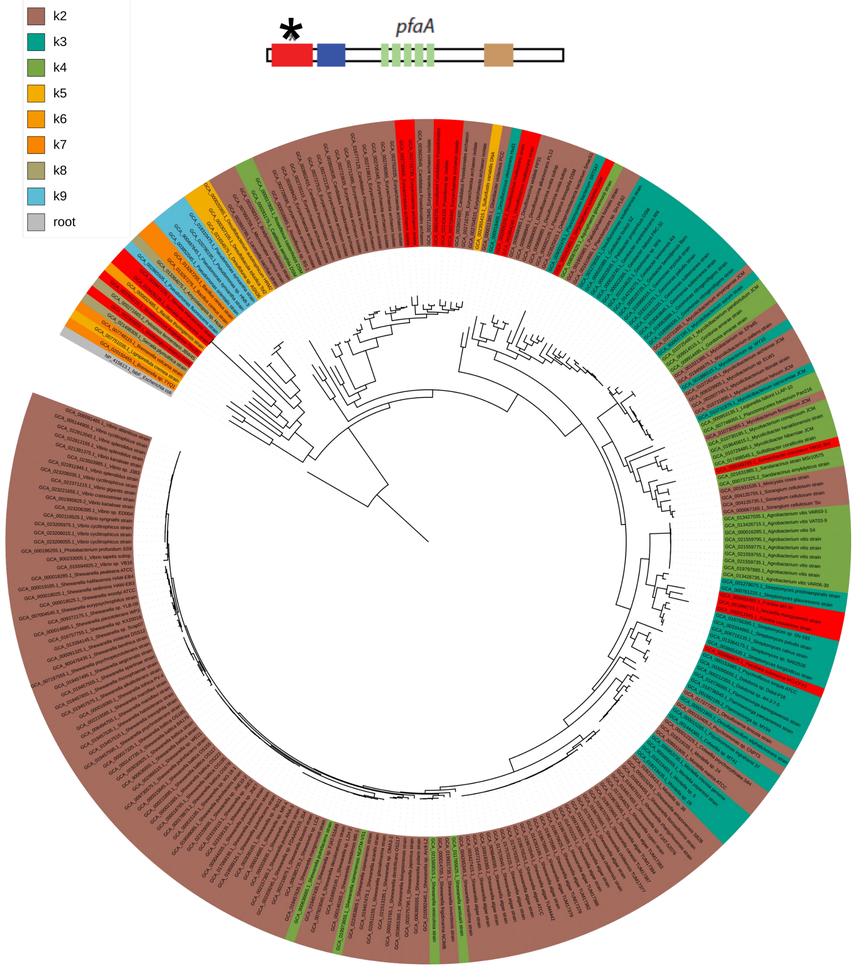

## Conclusions

The results support the idea that PUFA synthase complex is related to membrane fluidity, but rather so that molecules may rapidly move through the membrane, particularly proteins. In the case of *Shewanella*, we show here experimentally that despite EET mechanisms requiring membrane flux and renewal when exposed to insoluble ferric iron electron acceptors, cell cultures did not greatly express the PfaA-KS gene compared to cultures in a fully solubilized media. Informatics interrogation of the presently available fully sequenced prokaryote genomes indicates curious ***pfa*** synthase programs among phylogenetically divergent organisms likely related to environmental pressure.

## Phylogeny of PfaA-KS domain

The keto-acyl synthase (KS) domains from PKS cluster as a monophyletic group, allowing phylogenetic reconstruction of major lineages. Here the KS from the ***pfa*** clusters are used as the phylogenetic marker for GenBank whole genome data.

**262 GenBank organisms positive for putative PUFA synthases are widespread in deep lineages (*E. coli* fabF root). The domain architecture hierarchical clustering is represented on the tree by node colors**




The hierarchical cluster groups from pairwise distance of the domain architectures appear partly untethered from a phylogenetic model of the ***pfa*** synthase gene complex, indicating that prokaryotes may swap domains or co-opt traits as needed by environmental conditions. This lends evidence for an environmentally determined synthesis product.

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REWIRE Reinforcing Women in Research

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