Single cell genome reconstruction of two uncultured, proteorhodopsin-containing Flavobacteria

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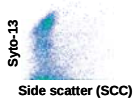
Abstract

Determining the genetic makeup of predominant microbial taxa with specific metabolic capabilities remains one the major challenges in microbial ecology and bioprospecting, due to the limitations of current cell culturing and metagenomic methods. The complexity of microbial communities and intraspecies variations hinders the assembly of individual genomes from metagenomic shotgun libraries. Here we report the use of single cell genomics to access the genome of two proteorhodopsin-encoding flavobacteria from Gulf of Maine bacterioplankton. We use high throughput fluorescence-activated sorting of single cells, whole genome amplification via multiple displacement amplification, PCR-screening and subsequent shotgun sequencing of these single amplified genomes (SAGs), allowing the genomic analysis of their novel photometabolic system and the sequence comparison to environmental marine sequence data.

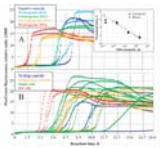
Background & Methods

Costal water (Maine)

Cell sorting



Real-time multiple displacement amplification (MDA) of single cells



16S rRNA gene and proteorhodopsin gene PCR of single cells

Stepanauskas, PNAS 2007.

Sequencing

	MS024-2A	MS024-3C
454 data	73 Mb	90 Mb
3Kb Sanger	4.58 Mb	5 Mb
8Kb Sanger	12.87 Mb	17.67 Mb

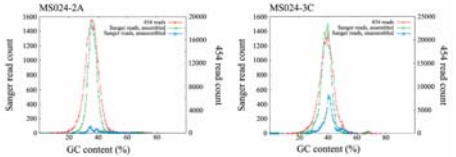
Sequence assemblies

General features of the flavobacterial SAG assemblies.

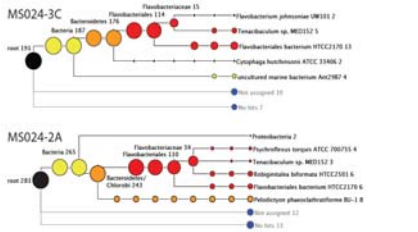
	MS024-2A	MS024-3C
Assembly statistics		
Assembly size [bp]	**1,905,484**	**1,515,248**
Estimated genome size [bp]	2,156,286 - 3,004,105	2,307,484 - 3,726,020
Number of contigs	**17**	**21**
Largest contig [bp]	684,032	549,383
GC content [%]	**36**	**39**
Mean read depth (± sd)	56 (± 63)	83 (± 110)
454 reads	**47**	**68**
Sanger reads	**9**	**14.3**
Gene predictions		
Total genes	**1,824**	**1,426**
Protein coding genes	1,785	1,400
with function prediction	**1,205**	**960**
w/o function prediction	**580**	**440**
Number of rRNA operons	2	1
Number of tRNA genes	**33**	**24**

The sequence data of the SAGs was Phrap assembled, followed by primer walking on shotgun clones, and PCR/adapter PCR on the diluted MDA products.

Data QC

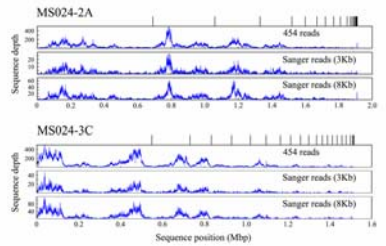


GC contents histogram of the unassembled and assembled Sanger and pyrosequence reads for the two SAG exhibits a tight uni-modal distribution.

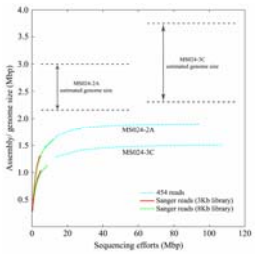


Taxonomic assignment analysis. The taxonomic contents of the blastx output for the unassembled reads of the Flavobacteria sp. MS024-2A was estimated and visualized using the Metagenome Analyzer (MEGAN) (Huson, Genome Res 2007).

The MDA bias



MDA bias as evaluated by sequence depth distribution. The contigs for the SAG are aligned by length and contig breaks are indicated by the tic marks along the top. The mean sequence depth is 56 (± 63) for MS024-2A and 83 (±110) for MS024-3C.



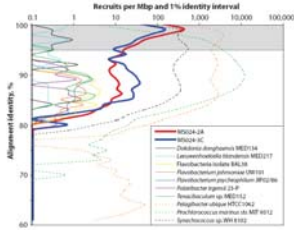
Genome coverage as function of the genome sequencing effort for the flavobacterial SAG. The curve displays near-saturation indicating that additional sequencing would mostly result in repeated sampling of the over-amplified genomic regions, not targeting the yet missing part of the genome.

Chimeras

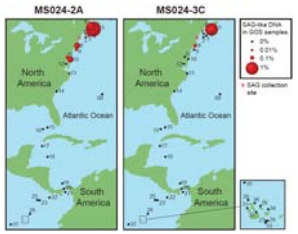
Table S1. Chimeric rearrangments in the SAG DNA.

	MS024-2A		MS024-3C	
	chimeric reads/ clones (%)	overall chimerism	chimeric reads/ clones (%)	overall chimerism
Read-based chimerism				
3Kb library reads (untreated MDA DNA)	1.9	1 chimer/ 28 Kbp	NA	NA
3Kb library reads (S1 treated MDA DNA)	2.0	1 chimer/ 25 Kbp	1.9	1 chimer/ 33 Kbp
8Kb library reads (S1 treated MDA DNA)	2.1	1 chimer/ 30 Kbp	1.6	1 chimer/ 40 Kbp
454 reads (S1 treated MDA DNA)		1 chimer/ 19 Kbp		1 chimer/ 25Kbp
Average (all reads)		**1 chimer/ 21 Kbp**		**1 chimer/ 27Kbp**
Clone-based chimerism				
3Kb clones (untreated MDA DNA)	14.5	1 chimer/ 20 Kbp	NA	NA
paired reads facing into the same direction	8.6			
paired reads facing away from insert	3.8			
paired reads outside the insert size range	0.2			
paired reads in different contigs	0.8			
paired reads contained in each other	1.1			
3Kb clones (S1 treated MDA DNA)	**16.8**	**1 chimer/ 15 Kbp**	**16.4**	**1 chimer/ 20 Kbp**
paired reads facing into the same direction	**7.8**		**9.6**	
paired reads facing away from insert	**3.0**		**0.9**	
paired reads outside the insert size range	**3.2**		**4.5**	
paired reads in different contigs	**2.0**		**0.8**	
paired reads contained in each other	**0.8**		**0.6**	
8Kb clones (S1 treated MDA DNA)	26.4	1 chimer/ 17Kbp	29.5	1 chimer/ 27Kbp
paired reads facing into the same direction	22.6		17.4	
paired reads facing away from insert	2.5		2.7	
paired reads outside the insert size range	7.4		3.9	
paired reads in different contigs	3.3		3.8	
paired reads contained in each other	0.6		1.8	
Average (all clones)		**1 chimer/ 17Kbp**		**1 chimer/ 23Kbp**

Fragment recruitment



Global Ocean Sampling (GOS) (Rusch, PLoS Biol 2007) metagenome fragment recruitment by the SAGs MS024-2A and MS024-3C, the currently sequenced marine Flavobacteria isolate genomes, the non-marine *F. johnsoniae*, and the three best GOS fragment recruiters *Pelagibacter*, *Prochlorococcus* and *Synechococcus*.



Geographic distribution of the GOS (Rusch, PLoS Biol 2007) metagenome fragments with >95% nucleotide identity to MS024-2A and MS024-3C.

Conclusion

Using the single cell approach, we demonstrate how a combination of single cell FACS and amplification via MDA can be used to access the genomes of uncultured environmental microorganisms, representative of their given environment.

Acknowledgements

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