

## MONO LAKE MICROBIAL DIVERSITY.

Please cite Humayoun et al. (2003) when referring to this information

The Mono Lake Microbial Observatory recently completed a survey of the phylogenetic diversity of Mono Lake bacteria (Humayoun et al., 2003). We analyzed the variation with depth in the composition of Bacteria in samples from the mixolimnion (2 m, aerobic), the base of the oxycline (17.5 m, microaerophilic), the upper chemocline (23 m, seasonally anoxic) and the monimolimnion (35 m, perennially anoxic and highly reducing). Composition was assessed by sequencing randomly selected, cloned fragments of 16S rRNA genes PCR-amplified from the DNA samples, which may have biased the outcome of our analysis (cf von Wintzingerode et al. (1997) and other papers).

<b>Table 1. Distribution of ribotypes in Mono Lake clone libraries.</b> See Humayoun et al. (2003) for details. Columns headed <b>Phylogeny</b> give: group, percent similarity to most similar sequence, accession number of most similar sequence. Columns headed <b>%</b> give the percentage of the clones in that library that were $\geq 97\%$ similar to this ribotype. ACT – Actinobacteria; CF – Cytophaga-Flexibacter; BC – low G+C Gram positive; SP – Spirochaeta; Unk – Unknown, no closely related sequence; $\alpha$ , $\beta$ , $\gamma$ – $\alpha$ -, $\beta$ - and $\gamma$ -Proteobacteria. Total: total percentage of clones in the library represented by the ribotypes listed in this table.							
<b>2 m Library</b>		<b>17.5 m Library</b>		<b>23 m Library</b>		<b>35 m Library</b>	
<b>Phylogeny</b>	<b>%</b>	<b>Phylogeny</b>	<b>%</b>	<b>Phylogeny</b>	<b>%</b>	<b>Phylogeny</b>	<b>%</b>
ACT, 92, AB038407	25	ACT, 96, X77443	43	$\alpha$ , 97, AF248638	10	CF, <80, Unk2	16
ACT, 96, X77443	19	ACT, 92, AB038407	18	BC, <80, Unk1	10	BC, <80, Unk1	11
$\gamma$ , 93, AJ404972	19	CF, 96, AF348716	10	ACT, 92, AB038407	7	BC, <80, Unk3	9
$\alpha$ , 97, AF248638	13	$\gamma$ , 98, AF016046	8	CF, <80, Unk1	7	BC, <80, Unk2	5
CF, 96, AF348716	8	$\gamma$ , 93, AJ404972	5	CF, <80, Unk2	7	BC, 86, AF203703	5
CF, 89, AB01526	6	ACT, 90, AF186411	3	SP, 93, X93927	7	CF, <80, Unk	4
ACT, 90, AF186411	4	$\alpha$ , 97, AF248638	3	$\gamma$ , 93, AJ404972	5	CF, <80, Unk4	4
BC, 83, AJ276353	4	$\beta$ , 94, AB046605	3	BC, <80, Unk2	5	CF, <80, Unk5	4
$\beta$ , 94, AB046605	2	$\gamma$ , 83, AF195410	3	$\alpha$ , <80, AJ244796	2	BC, 82, AF266461	4
<b>Total</b>	<b>100</b>		<b>93</b>		<b>61</b>		<b>62</b>

Most of the 212 sequences retrieved (ca 60 clones were analyzed per sample) fell into 5 major lineages of the domain Bacteria:  $\alpha$ - and  $\gamma$ -Proteobacteria (6% and 10%, respectively); Cytophaga-Flexibacter (CF, 19%); high G+C Gram positive (actinobacteria, 25%); and low G+C Gram positive (Bacillus/Clostridium, 19%). Twelve percent were identified as chloroplasts. The remaining 9% represented  $\beta$ - and  $\delta$ -Proteobacteria; Verrucomicrobiales, Planctomycetales and candidate divisions. The populations of sequences retrieved from mixolimnion and oxycline libraries were dominated by sequences related to actinobacteria (49 and 63%, respectively) distributed into only 3 distinct ribotypes, (defined as sequence similarities  $\geq 97\%$ , most were  $\geq 99\%$ ), while the population of sequences retrieved from the monimolimnion library was dominated (52%) by low G+C Gram positive bacteria distributed in 12 distinct ribotypes. We have not been able to retrieve archaeal ribotypes from these samples using published domain Archaea primers.

A standard coverage estimate,  $C=1-(n_1/N)$ , where  $n_1$  is the number of ribotypes that occurred only once in the clone library and  $N$  is the total number of clones analyzed (Good, 1953) indicated that we sampled 59-98% of the diversity in our libraries by sequencing ca 60 clones. Mixolimnion and oxycline libraries had low sequence diversity with only 9 and 12 distinct ribotypes respectively; whereas chemocline and monimolimnion libraries were more diverse, containing 27 and 25 ribotypes, respectively. The composition of mixolimnion and oxycline libraries were not significantly different (LIBSHUFF analysis; Singleton et al., 2001), but they were significantly different from chemocline and monimolimnion libraries ( $p < 0.001$ ) and chemocline and monimolimnion libraries were not significantly different from each other ( $p = 0.006/0.124$ ).

We used the distribution of ribotypes in our clone libraries to estimate the total number of ribotypes present in the samples using an analytical approach described by (Curtis et al., 2002). This analysis indicated that the total number of different ribotypes per sample ranged from 200 (17.5 m sample) to 4700 (23 m sample). The number of species in oceanic plankton samples or soil samples estimated by the same (Curtis et al., 2002) or other (Torsvik et al., 1990; Torsvik et al., 1996; Torsvik et al., 2002) methods is of the order of 10's of thousands.

These species are not all equally abundant. Our data indicate that Mono Lake libraries are strongly dominated by a few ribotypes (Table 1). All of the clones from the 2 m sample could be assigned to just 9 ribotypes, with the 3 most abundant ribotypes accounting for 63% of all sequences. Forty three percent of the clones in the 17.5 m library fell into one ribotype while the 3 most abundant ribotypes accounted for 73% of the sequence population. The most abundant ribotype in the most diverse library (23 m) accounted for 10% of the population while the 3 most abundant ribotypes in this library accounted for 27% of the sequence population.

## Literature Cited

- Curtis, T.P., Sloan, W.T., and Scannell, J.W. (2002) Estimating prokaryotic diversity and its limits. *Proceedings of the National Academies of the U.S.* **99**: 10494-10499.
- Good, I.J. (1953) The population frequencies of species and the estimation of the population parameters. *Biometrika* **40**: 237-264.
- Humayoun, S.B., Bano, N., and Hollibaugh, J.T. (2003) Depth distribution of microbial diversity in Mono Lake, a meromictic soda lake in California. *Applied and Environmental Microbiology* **69**: 1030-1042.
- Singleton, D.R., Furlong, M.A., Rathbun, S.L., and Whitman, W.B. (2001) Quantitative comparisons of 16S rRNA gene sequence libraries from environmental samples. *Applied and Environmental Microbiology* **67**: 4374-4376.
- Torsvik, V., Goksoyr, J., and Daae, F.L. (1990) High diversity in DNA of soil bacteria. *Applied and Environmental Microbiology* **56**: 782-787.
- Torsvik, V., Sorheim, R., and Goksoyr, J. (1996) Total bacterial diversity in soil and sediment communities - A review. *Journal of Industrial Microbiology* **17**: 170-178.
- Torsvik, V., Ovreas, L., and Thingstad, T.F. (2002) Prokaryotic diversity--magnitude, dynamics, and controlling factors. *Science* **296**: 1064-1066.
- von Wintzingerode, F., Göbel, U.B., and Stackebrandt, E. (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiology Reviews* **21**: 213-329.