

S1 Appendix: Evolution of cross-tolerance to metals in yeast

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GITHUB address: https://github.com/joelkcamp/crossTolerance_bazzicalupoEtAl

1 Tolerance curves

To decide which concentrations to use in our experiment we characterized the tolerance curves of the yeast strain W303 and the BY4741. We used W303 in our evolution experiment. BY4741 had been used previously and we characterized its tolerance curves to several metals.

R script to extract growth curve information: 1_shape_clean.R. This script calculates the spline using the max_growth_rate.R and bioscreen_functions.R, and summarizes the findings into tolerance curves.

2 Reciprocal Transplant Growth Curves

Once the yeast lines were evolved, we performed a reciprocal transplant experiment. Similar to the tolerance curves, this script extracts the information for all the bioscreen OD readings:

2_evo_test.Rmd

We inspected growth curves and matched the expects ancestor in YPAD to perform and match our expectation and controls were clean.

Compromised batch folder contains a machine (2 bioscreen plates) that did not show growth in the ancestor in permissive media and we excluded it from further analyses because it's not clear if it worked properly.

3 Reciprocal Transplant Cross Tolerance

We calculate cross-tolerance in a broad and a narrow sense and test the amount of cross tolerance against different genomic aspects of the evolved lines. Script: 3_cross_tol.Rmd

4 Genome Analysis

We sequenced the genomes of 109 lines + ancestor. Steps from .fastq files to .vcf variant calling are included in folder: /4_bash

5 Petite Analysis

We recorded petite phenotype and we looked at the performance of the lines in each metal. There was some hint that perhaps it may be advantageous and adaptive to be petite in cobalt and manganese where almost all lines evolved a petite phenotype. We tested petite and grande lines

from a different experiment using the same genetic background strain and showed that it is not the case and being petite in itself does not help in those metals.

We also report the coverage of the mitochondrial genome for these lines. We used the mpileup program on bam files (see bash folder for mpileup script). A custom made perl script calculates the coverage for 100 base pair windows (see depth folder for the windows.pl script to calculate the average coverage in windows). The Rmd script uses the output of the perl script to plot the coverage. Most of the manganese and one copper and two nickel lines showed increased coverage in previously described breakpoints. All cobalt lines and most of the copper petite lines completely lost the mitochondrial genome.

Script 5_metal_petite.Rmd

6 Chromosome Duplication

We found whole chromosome duplications by estimating coverage with our custom-made perl script calculates the coverage for 1000 base pair windows (see depth folder for the windows.pl script to calculate the average coverage in windows). The Rmd script uses the output of the perl script to plot the coverage of chromosomes. The results are summarized in a table.

We also estimated the relative copy number of the gene CUP by following Gerstein et al. (2015)'s protocol. In folder 'depth'.

Script: 6_aneuploidy.Rmd

7 SNP cleaning

We performed SNP cleaning in Mathematica and R.

7_snp_analysis_final_18Nov2024.Rmd

BazzicalupoSupMat_FinalizingGenes8Nov2024.nb

8 GO Terms

The gene annotations for the SNP mutations we are confident in were used to predict cellular component and biological process GO terms in the SGD. We then compared the proportion of each of the GO terms between the genes we found and the whole genome using a Fisher's Exact test to find terms significantly over-represented.

Script: 8_GO_analysis_11Nov2024.Rmd

9 Predictions

To find ways to predict cross tolerance we used different information about the environment and genes known to matter for metal tolerance to run a random effects model. We first analyzed the potentially predictive data, so the scripts in the following subsections were used first.

Script: 9_predictions.Rmd

63 **9.1 SGD**

64 We calculated an expected amount of cross-tolerance based on the overlap of known genetic muta-
65 tions conferring cross-tolerance between pairs of metals.

66 Script: predictions/SGD_metal_resistance_genes_clean.Rmd

67 **9.2 Electropotential and Parts Per Million**

68 We measured the Oxidative Reductive Potential of the media we used in the experiment and the
69 amount in parts per million of the metals to predict how similar the stress between metals is and
70 how much metal the yeast are exposed to in the regular growth medium.

71 Script: predictions/electrode_potential.Rmd

72 **9.3 US Geological Survey**

73 We downloaded ppm of metals in soils and computed correlations for all pairs of metals to obtain
74 a measure of how often they co-occur in the environment.

75 Script: predictions/soil_metals.Rmd

76 **10 snpEff**

77 SNP mutations were annotated with snpEff and the output of the programme was manipulated
78 with this script. Script: snpEff.Rmd

79 snpEff_PDBupdate.Rmd

80 **11 Spectra**

81 We compared the types of mutations in our experiment with the mutational spectra measured in
82 Lynch et al. (Table 1 in their manuscript) from their mutation accumulation experiment. We also
83 included results from Gerstein et al. as a control and repeat their test. The mutational spectra were
84 extracted for each metal from the snpEff annotation output. Script: mutationtypes.Rmd