Investigation of the function of mutated gene in different environmental challeng...

Science, Genetics

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ABSTRACT

Genome wide analysis of haploid yeast knockout mutants with phenomics applications which plays important role in sensing the stress caused by environmental perturbations and allowed the identifications of those phenotypes which adapt to environmental challenge.

INTRODUCTION

Phenomics evaluation of individuals carrying novel mutation represents a path of investigation to further elucidate the function of mutated gene in different environmental challenges observed on microtitre plate. Numerous functional genomic approaches have been developed to study the model organism yeast, Saccharomyces Cerevisiae, with aim of understanding the biology of cell. Some of these techniques are based on yeast growth differences under different conditions such as those generated by gene mutation, chemicals or both. [Memacian et al. (2007)]. Phenomics is systematic measurement and analysis of qualitative and quantitative traits including imaging methodologies, for refinement and characterization of

phenotype. In phenomics study an array of technologies of mutants lacking each one gene has been applied in microbial growth on solid media [Blomberg 2001] which provides better cost efficiency. Collection of artificial gene constructs e. g., Single gene knockouts, have facilitated gene function analysis in lab (Giaever et. al. 2002).

MATERIALS AND METHODS

Synthetic genetic array analysis was conducted using colony arrays corresponding to single yeast gene knockouts each containing 1536 colonies with wild type control colonies in every fourth position and three replicates of each strain in juxta position for confirmation of experiment. The mutant collections were subjected to basal medium and (NaCl) salt medium and growth of yeast was identified in different medium using Scan-o-Matic. Plates without lids are fixed in custom-made acrylic glass fixtures position, it captures four plates per image. Scanners were maintained in room temperature environment in a thermostat controlled room and kept covered with boxes, preventing inflow of light. Scan-o-Matic recorded and analysed 100, 000 growth curves in parallel every 20 minutes being the minimum time interval for each scan and colony size data was extracted with high accuracy and high throughput resolution. Scan–o-Matic is written in python 2. 7. Matplotlib is used for graph production.

Images with growth curves were used for computation and analysis. Colony population size was extracted from raw images is a multi-step procedure image acquisition, colony detection, background deletion and pixel intensity estimation. Raw growth curves produced by Scan-o-Matic were smoothed to

reduce the influence of noise by using Mean filter which removed local spikes. To maximize standardization and reproducibility the quantitative growth measures in a particular environment were normalized to the average response of one wild type included in each run. This data was further processed using R to finally get mutants with differing growth phenotypes. In R, Gene-Environment Interaction was performed to get sensitive and salt resistant colonies by subtracting growth defects rate in NaCl media and without NaCl medis(Basal) then t test was performed and obtained p values were adjusted by FDR (False Discovering Rate). The colonies with strongest interactions were further analysed.

RESULTS

Analysing the growth of 43, 008 viable haploid deletion strains and wild type yeast provided systematic high resolution growth curves for a complete mutant collection. With high precision we quantified the growth characteristics lag phase (time to adapt to environmental challenge), growth rate (generation time), yield (efficiency of growth). It was found that many strains with a defect in growth in salt medium also showed same defect in basal medium. To distinguish the specific salt growth defect in deletion strain from general growth defect, basal and salt medium were combined to know the interactions providing a sensitivity measure for each strain to salt.