



ELSEVIER

Application of phenotypic microarrays to environmental microbiology

Sharon Borglin¹, Dominique Joyner¹, Kristen M DeAngelis^{1,2,3},
Jane Khudyakov³, Patrik D'haeseleer^{3,4}, Marcin P Joachimiak^{5,6} and
Terry Hazen^{1,3,7,8}

Environmental organisms are extremely diverse and only a small fraction has been successfully cultured in the laboratory. Culture in micro wells provides a method for rapid screening of a wide variety of growth conditions and commercially available plates contain a large number of substrates, nutrient sources, and inhibitors, which can provide an assessment of the phenotype of an organism. This review describes applications of phenotype arrays to anaerobic and thermophilic microorganisms, use of the plates in stress response studies, in development of culture media for newly discovered strains, and for assessment of phenotype of environmental communities. Also discussed are considerations and challenges in data interpretation and visualization, including data normalization, statistics, and curve fitting.

Addresses

¹ Ecology Department, Earth Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, MS 70A-3317, Berkeley, CA 94720, USA

² Microbiology Department, University of Massachusetts, Amherst, MA, USA

³ Microbial Communities Group, Joint Bioenergy Institute, Emeryville, CA, USA

⁴ Biosciences and Biotechnology Division, Lawrence Livermore National Laboratory, Livermore, CA, USA

⁵ Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

⁶ Virtual Institute of Microbial Stress and Survival, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

⁷ Department of Civil and Environmental Engineering, University of Tennessee, 676 Dabney Hall, Knoxville, TN, 37996-1605, USA

⁸ Oak Ridge National Laboratory, Biosciences Division, Oak Ridge, TN, USA

Corresponding author: Hazen, Terry (tchazen@utk.edu)

Current Opinion in Biotechnology 2012, **23**:41–48

This review comes from a themed issue on
Analytical biotechnology
Edited by Wei E. Huang and Jizhong Zhou

Available online 2nd January 2012

0958-1669/\$ – see front matter
Published by Elsevier Ltd.

DOI [10.1016/j.copbio.2011.12.006](https://doi.org/10.1016/j.copbio.2011.12.006)

Introduction

Growth conditions for microbes are as diverse as the environments they inhabit. Microbes have been found in all investigated environments so far, which include

extremes in pH, redox potential, substrate concentrations, pressure, and temperature. Owing to the sometimes extremely specific growth requirements of microbes, the use of multiwell plates for cultivation of distinct organisms has become well-established for both bacteria and fungi encompassing a wide array of applications in the environmental, food safety, and medical fields.

Phenotypic microarray technology is growth of microorganisms, either pure culture, community, or consortia, in multi-well plate each with a different test component in each well, enabling a screen of the phenotypic characteristics of the test culture. This technology allows for culturing in small volumes providing opportunity to perform many parallel assays in a compact space. Examples of well components could include but are not limited to C,N,P,S-sources, amino acids, pH or NaCl tolerance, or antibiotic resistance. A phenotypic microarray is commercially available through Biolog, Inc. (Hayward, CA), which provides a suite of twenty 96-well plates, each containing a different substrate, stressor, or nutrient [1,2]. This system works by culturing cells either aerobically or anaerobically [3,4], and assaying growth colorimetrically on a microplate reader or the Omnilog unit (Biolog, Hayward, CA). By comparing growth in these plates, phenotypic changes can be detected as strains and environmental conditions are varied. The plates can also be user prepared, giving greater control and knowledge of media components.

Key advances in the last few years have extended the application of growth phenotype arrays to organisms with special growth requirements. Several publications in recent years have described novel applications of phenotypic microarrays that pave the way towards understanding environmental microbiology. Some of the most promising applications lie in defining changes in phenotypes as a result of genetic manipulation and screening for possible growth conditions for uncultured organisms. Several publications have discussed the use of phenotypic microarrays to assess phenotypes of knockout mutants [5*,6*,7,8,9**,10] or investigation of chemotactic response [11*]. Other applications use microplates to investigate phenotypic changes owing to different environmental conditions such as temperature, antibiotic resistance, or other stressors [12*,13**,14,15*]. Growth microarrays have also been used to differentiate function between strains of the same species [16]. These applications emphasize the need to confirm genetic predictions with phenotypic profiling.

In this review, several novel applications of the phenotypic microarrays are described, along with particular method modifications needed. Most current applications require the development of proper inoculation, growth conditions, and assay methods, which are necessary for reproducible and meaningful results. Also included is a discussion of current data visualization and data processing techniques, both of which present challenges encountered when interpreting PM data.

Anaerobic growth

The PM system was originally designed to profile metabolic activity of aerobic organisms. To assay phenotypes during aerobic metabolism a tetrazolium salt is used that is reduced by dehydrogenases and reductases produced by cells to yield a formazan dye, indicating that the inoculated organism are actively metabolizing a substrate in the well. No color change implies that the cells are not active and the substrate of interest, for example, a specific carbon source, is not metabolized. For anaerobes, reduction of the medium is often required and the lower reduction potential of the media results in abiotic transformation of the tetrazolium salts. However, studies have demonstrated that formazan dyes can be utilized to measure activity in growth assays under anaerobic conditions [17,18] if the organism of interest can grow in non-reduced medium or if the added reductant does not lower the redox potential below that required to transform the indicator dye. However in these cases it is possible the dyes will also not be reduced by the activity of the metabolizing organism and it may become necessary to add metabolic boosters such as yeast extract and increased inoculum concentration to measure activity of the organism [17,19]. Care should be taken when choosing the type of dye used, as some measure inter-cellular and others extra cellular activity, formazan produced can be water soluble or insoluble, and some dyes can cause cell death on transformation [20].

Since the Omnilog detector measures opacity, alternatives can be found to dyes, usually by finding an indicator of activity that increases opacity. For the anaerobic sulfate reducer *Desulfovibrio vulgaris* (DvH) the medium was reduced with titanium citrate resulting in an electric potential of approximately -400 mV, which reduced all available tetrazolium salts. In this case the chemistry of DvH provides a useful indicator of metabolic activity on a substrate: a byproduct of sulfate reduction is the generation of hydrogen sulfide that complexes with iron and other metals in the medium to form metal sulfide precipitates [21]. These blackened precipitates substitute for the redox dye as an indicator of metabolic activity in the wells. For this organism, PM panels were assayed in an anaerobic environment on the PM by first inoculating panels in an anaerobic chamber and heat-sealing the inoculated panels into fitted gas impermeable bags [3]. Once sealed the bags can be transferred outside of the

chamber and incubated anaerobically. The gas and plate filled bags are sealed in place to be read on the PM with a simple engineering of the trays with clamps.

When using byproducts of growth, such as precipitates generated by DvH or color change of the tetrazolium dyes, it must be noted that the assay is a measure of sulfate reduction or other metabolic activity, which is possibly a byproduct of growth [22,23]. In addition there can be significant color change detected by reactions that can and do occur abiotically by some of the chemical agents added to the PM wells [3]. This must be taken into account when discussing a kinetic plot as they are not all comparable to traditional growth curves. Abiotic reactions can be assayed by filling wells with sterile or heat-killed medium.

Alternatively, other assays of growth or metabolism can be used. With the iron reducing anaerobe *Geobacter metallireducens* a metabolic dye could not be used as it was reduced by the medium and the turbidity of the final biomass yield was too low to be detected by the Omnilog optics [24]. For this Fe reducer, the metabolism of the organism on substrates of interest was measured by a colorimetric method utilizing ferrozine to quantify reduced iron in the wells of the PM plate. With this method, the PM plates were inoculated and incubated, but not recorded by the data logging system. Each well was assayed destructively after incubating for a prescribed amount of time. In this case the Omnilog can only be used as an endpoint detector. These assays could potentially be made high throughput by coupling the Omnilog with robotics systems (see Figure 1).

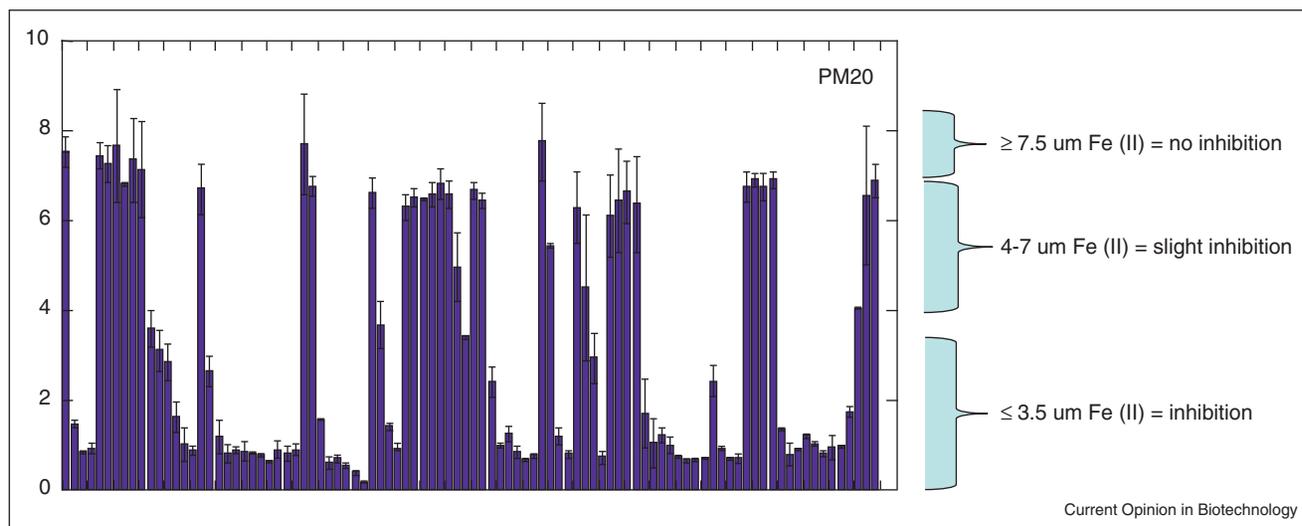
Thermophilic growth

The Omnilog instrument has an operating incubating temperature with a maximum of 45°C . In the case of hyperthermophiles such as *Sulfolobus sulfotarius*, high temperature phenotypic profiles were generated using the PM array in a specialized incubator. Plates were incubated at 80°C without deformation of plastic or destabilization of the dye. It is essential that the panels be placed inside a humidified chamber to prevent evaporation. Activity was then logged as an endpoint or over time on the Omnilog or other plate reader by removing the plate from the high temperature incubator and placing onto data logger for each read.

Media development

Based on phylogenetic marker sequences, the diversity of known bacteria and archaea is vast. There are an estimated 10^5 to 10^9 distinct microbial species or more [25,26] and only about 11,000 have been described and named [27,28]. However, in order to understand metabolic function, regulation, and true diversity, it is necessary to be able to cultivate microbes. Despite new techniques that are constantly being developed to increase the number of

Figure 1

Growth response of *Geobacter metallireducens* on PM 20 plate using the ferrozine assay.

bacteria and archaea in cultivation [29,30], and consolidated efforts to accumulate more genomic information are being undertaken, such as the Genomic Encyclopedia of Bacteria and Archaea (GEBA) project [29], only a relatively small fraction, 0.1–1% are cultivated [25]. Though many species will probably continue to resist cultivation, large-scale screening of media conditions can help bring microcolonies or slow-growing organisms into cultivation at concentrations high enough for study. A massively parallel screen such as the Biolog platform offers a fast and thorough way of developing custom defined media to improve growth. In order to empirically determine optimal growth conditions for the novel isolate *Enterobacter lignolyticus* SCF1, phenotypic arrays were performed iteratively [31,32]. Starting with the media that the strain was isolated on, elements were substituted out (carbon, nitrogen, phosphorous, sulfur); after each optimum nutrient was determined, that nutrient was substituted into the next plate. Data from the PM array were able to demonstrate more robust growth on D-fructose and to determine that the strain favored glycine–glutamine (Gly–Gln) over ammonia.

Stress response

The ability of PM plates to measure growth rate for hundreds of strains in parallel presents an opportunity for screening single strains under multiple growth conditions using custom plates [33,34]. The MT ('empty') plate mode has been employed to test the response of individual isolates to conditions of increasing stress, for example, in the form of ionic liquids. Ionic liquids are being investigated for their ability to dissolve lignocellulose, with the application to pretreatment of feedstock plant materials as a first step in converting biomass to

biofuel [35,36]. As molten salts that affect the osmotic pressure of cells growing in the pretreatment medium, they are toxic to many laboratory strains [37] and therefore identifying host organisms that are resistant to ionic liquid stress is highly desirable [38].

Comparing Biolog growth curves of isolates tested with increasing IL concentrations, screening different ILs used for pretreatment, and testing other stress conditions such as increasing salt concentrations facilitates an efficient high-throughput approach for analyzing effects of ILs on microbial respiration and growth. By incubating parallel cultures in microplates we found that increasing concentrations of ILs 1-ethyl-3-methyl imidazolium chloride (abbreviated as [C₂mim]Cl) and [C₂mim]OAc affected the growth of isolate *E. lignolyticus* SCF1 by extending lag phase, decreasing log phase growth rate, and reducing final biomass yield owing to premature exit into stationary phase [31]. We were able to define that SCF1 can tolerate significant growth in 0.5 M [C₂mim]Cl and 1 M of salts such as NaCl, KCl, NaOH, and KOH, and that the IL stress response was distinct from the response to salt. Moreover, we found that growth measured by the Omnilog instrument was comparable to optical density-based growth measurements [31]. While some studies have used Biolog phenotypic microarray for in-depth comparison and metabolomic profiling of microorganisms exposed to stressors such as metals [33,39], we believe that this system is likewise applicable to fast, high-throughput screening for chemical toxicity effects on microbial growth and for identification of stress-tolerant, industry-relevant strains that can grow in the presence of ionic liquids, organic solvents, acids, bases, salts, or alcohols, among many others.

Oil spill consortia/isolate characterization

Another novel application of the PM is in the investigation of overall metabolism of microbial communities. We have collected the carbon source utilization profiles of two consortia from environments that were affected by the Macondo oil spill in the Gulf of Mexico [36]. These two consortia were enrichments from the deep ocean oil plume and from oil contaminated beach sand from the nearby coast. Comparison of the carbon source profiles reflects differences in the carbon metabolism of the two communities and gave insight into the carbon substrates to be utilized for the culturing of isolates from these different environments. In addition we supplemented the consortia with Macondo oil and dispersant to determine if this addition would elicit changes in the profiles. As expected, this PM data clusters by community (Figure 2), however the analysis highlights the unique and shared sets of functional capabilities in these two communities, a subject of further investigation. The metabolism of the consortia enrichments was not perturbed by the addition of hydrocarbons from the oil or dispersant, suggesting that these two communities were well adapted to the influx of these hydrocarbons owing to natural seeps in the environment. Future work includes comparing these results to microbial community and gene expression profiles from these environments [36,40]. Some challenges for community growth is choice of proper growth media, opaqueness that is not related to growth, and challenges that apply to all laboratory culturing owing to a microwell that is far removed from the natural environment of the community. In addition, the technology is limited to cells grown in liquid culture at standard pressure, which provides challenges when extending results to soil, sediment, or deep ocean environments.

Data analysis and visualization

Data normalization

Results from phenotype microarray measurements can be susceptible to experimental and biological noise as well as systematic errors and biases. Thus analysis of PM data may benefit from targeting removal and normalization of undesirable data features. For example, PM studies, like other micro-plate or small media volume experiments, are sensitive to the starting number of viable cells in the inoculum. It is challenging to provide viable cell counts in the inoculum and it is known that the initial cell count can significantly affect various growth parameter estimations from PM results [3]. Therefore a standard step in PM analysis should be to subtract the initial colorimetric measurement from all subsequent values in the PM growth curves. In cases where the initial cell count is expected to vary or the inoculum is expected to be heterogeneous, further corrections of the data may be necessary. To correct for systematic errors and biases, for example, media effects on the colorimetric measurement, control PM data with no inoculum should be collected and subtracted from the cell

growth samples. Furthermore, spatial effects have been observed to contribute to the data variance in microplate assays hence it may be beneficial to perform additional spatial normalizations [41,42]. Finally, standardization is used to place data from different wells of a PM plate on a common scale, for example, dividing by the mean of all wells [43]. To facilitate the biological interpretation of the data, if a relevant control experiment is possible, and then log-ratio values can be computed between the treatment cases and no treatment or between mutants and WT, for example. These operations on the data may be performed on the summary values computed from PM growth curves (e.g. the midpoint), on curve fitting parameters, or directly on the curves themselves. It is recommended to collect data on biological replicates as analysis of replicates gives a measure of experiment quality and can be used to compute confidence values for conclusions drawn from PM data. Some challenges in data normalization remain, such as how to account for biologically mediated interference with the colorimetric growth assays, how to identify failed or suspicious PM growth experiments, and how to preserve or accentuate informative features of growth curves. As more PM datasets accrue it should be possible to develop more robust normalization techniques based on distributions of values computed from compendia of PM experiments, perhaps analogously to Robust Multichip Average (RMA) normalization for gene expression microarrays [44].

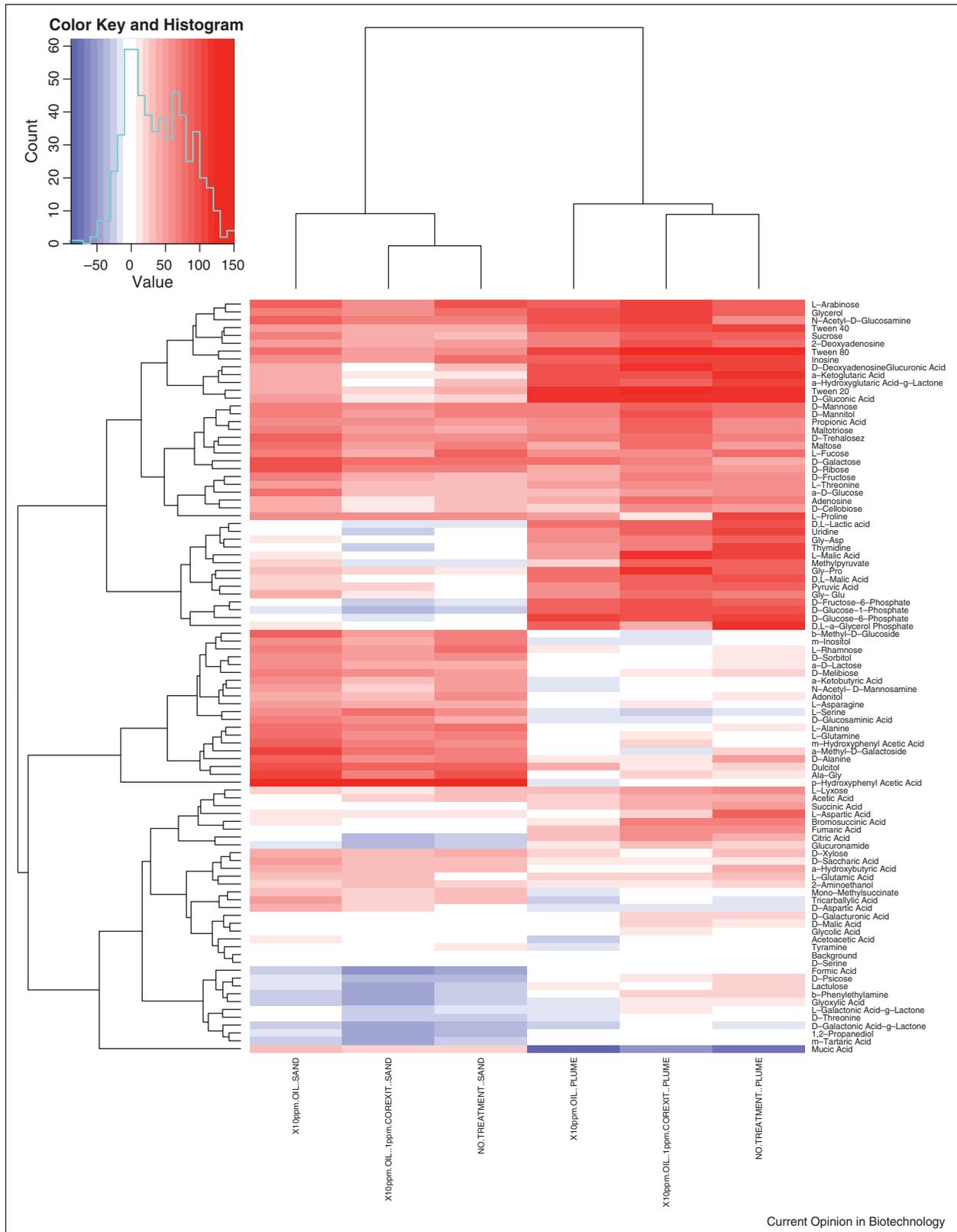
Statistical analysis and visualization

Once the raw PM data has been processed and normalized the resulting matrix of sample-by-growth condition values can serve as input for a variety of supervised and unsupervised statistical data analysis. Cluster heatmaps are one effective way to compute and visualize similarities in data, providing information on the relationships between PM growth conditions as well as samples. Such exploratory analysis aid in confirming hypothesis, in the identification of outliers, and can provide biological insight based on the resulting data clusters. These and other analysis are available, for example, in the multi experiment viewer MeV [45] as well as a number of packages in R and MatLab. Significance of the clustering can be assessed using silhouette analysis [46], permutation tests, or by bootstrapping [47] through resampling the growth condition data for sample clustering as well as resampling the sample data for growth condition clustering. A variety of statistical analyses have been proposed for phenotype microarray data [48,49] and the choice of method depends on the specific application. However, a phenotype microarray data evaluation standard and reference sets are needed by which to compare these methods as well as the methods for estimating growth curve parameters.

Curve fitting

Given the high throughput nature of the phenotype microarray technology, and the amount of detail captured in the shape of the resulting growth curves, it is surprising

Figure 2



This cluster heatmap was generated using the R ggplot2 package with Euclidean distance as the similarity measure and hierarchical clustering with complete linkage.

that many phenotype microarray analyses to date are based on qualitative comparisons of growth curves, or based on thresholding on summary values such as maximum response, average response or total area under the curve. The latter two parameters are equivalent, but are dependent on the length of the incubation. Average response or area under the curve also cannot distinguish between growth curves with a rapid rise but a low maximum response level, a long lag time followed by a rapid rise to a high maximum response level, or a slow but continuous increase to a high level, even though these are quite distinct phenotypes that one might want to distinguish.

Rather than condensing the entire growth curve into a single summary value, its most salient features can be captured by fitting a well-chosen parametric curve, either to the absolute or (more commonly) the log transformed PM data. . . Curve fitting has been used for community level physiological profiling (CLPP) of microbial communities based on carbon source utilization patterns [43,50,51], although less frequently for modeling PM growth curves of isolates [51–53]. The two most commonly used and best fitting models are Gompertz and the logistic model, both of which are sigmoidal models with parameters to capture the initial lag time λ , maximum growth rate μ_m , and asymptotic response. Zwietering *et al.* [54^{*}] compared these and other models to fit 40 growth curves of *L. plantarum* at different temperatures, and showed that the Gompertz equation provided a better fit than the logistic equation for most growth curves. DeNittis *et al.* [51] compared both models to fit 37 curves of average well color development (AWCD) across Biolog YT microplates for yeast isolates and mixed communities, and showed that the logistic equation slightly outperformed Gompertz in this case. While the logistic curve is symmetrical about the midpoint $t_{0.5}$, the Gompertz curve slopes more gently at the upper than the lower part of the curve (see Figure 3), which may be a more appropriate model for single-well growth curves.

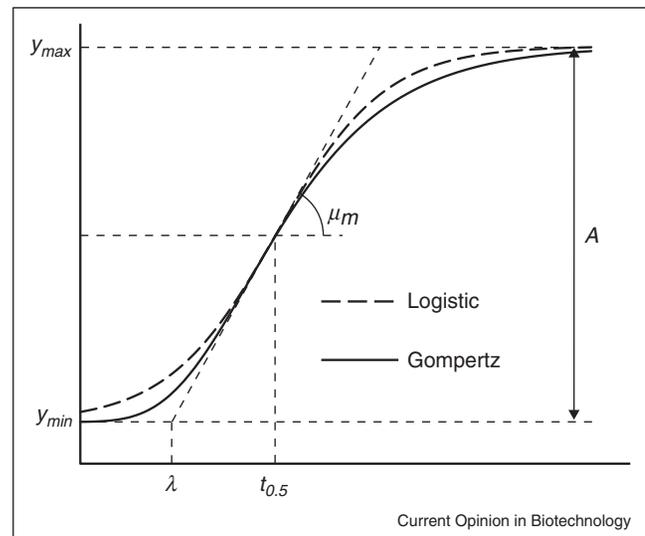
Modifying the formulation proposed by Zwietering [54^{*}] to include a lower asymptotic response level y_{\min} and an upper asymptote y_{\max} we get the following equations:

$$y(t) = y_{\min} + A / \left\{ 1 + \exp \left[\frac{4\mu_m}{A} (\lambda - t) + 2 \right] \right\} \quad \text{Logistic}$$

$$y(t) = y_{\min} + A \exp \left\{ -\exp \left[\frac{\mu_m e}{A} (\lambda - t) + 1 \right] \right\} \quad \text{Gompertz}$$

The time to reach the midpoint between y_{\min} and y_{\max} is typically much easier to estimate from the growth curve than the lag time λ , and is related to the above parameters by $t_{0.5} = \lambda + A/2\mu_m$. Both models can be fitted to growth curves in each well using standard nonlinear optimization algorithms, such as the `nlme()` function in the R statistical environment (<http://www.r-project.org>), and data from

Figure 3



Growth curve parameters for the Logistic (dashed) and Gompertz (solid) model.

replicates can be used to define confidence intervals on the parameter estimates, and test for significant changes in the parameters for the growth curves [52]. Measures of fit to the curve, or unusual parameter combinations can be used to filter out anomalous growth curves.

An alternative to parametric curve fitting is to represent the growth curve based on a few points on the curve. For example, one could estimate y_{\min} , y_{\max} , $t_{0.25}$, $t_{0.5}$, and $t_{0.75}$ directly from the growth curve data, and these values will define the curve fairly accurately. However, care must be taken to filter out any noise that may bias these point estimates, whereas the curve fitting automatically averages across the entire growth curve.

Conclusions

Phenotypic profiling is an essential step for understanding genotype differences, stress response, media design, and changes in environmental conditions for environmental microorganisms of interest in contaminant remediation, biofuels production, and climate change. Each new type of organism presents a challenge to adapt PM technology to accurately reflect changes in growth owing to different substrates and stressors in the plate. Future needs include extending the technique to extremophiles, cyanobacteria and algae, and archaea, and also to establish methods of comparison between strains and mutants owing to differences in backgrounds, growth rate, and media. It should be noted that the proprietary nature of the Biolog[®] plate contents makes precise determinations of proper inhibitory concentrations and media components difficult. In addition in some cases the concentrations of added nutrients may be too low or in a form that is unavailable to the

target organism, especially as environmental conditions such as temperature and atmosphere are modified.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Bochner BRPG, Panomitros E: **Phenotype MicroArrays for high-throughput phenotypic testing and assay of gene function.** *Genome Res* 2001, **11**:1125-1246.
 2. Bochner B: **New technologies to assess genotype-phenotype relationships.** *Nat Rev Genet* 2003, **4**:309-314.
 3. Borglin S, Joyner D, Jacobsen J, Mukhopadhyay A, Hazen TC: **Overcoming the anaerobic hurdle in phenotypic microarrays: Generation and visualization of growth curve data for *Desulfovibrio vulgaris* Hildenborough.** *J Microbiol Methods* 2009, **76**:159-168.
 4. Koutny M, Zaoralkova L: **Miniatured kinetic growth inhibition assay with denitrifying bacteria *Paracoccus denitrificans*.** *Chemosphere* 2005, **60**:49-54.
 5. Johnson DA, Tetu SG, Phillippy K, Chen J, Ren Q, Paulsen IT: **High-throughput phenotypic characterization of *Pseudomonas aeruginosa* membrane transport genes.** *PLoS Genet* 2008, **4**:e1000211.
Used phenotype array to identify phenotype changes in knockout mutants, focusing on transporter genes. Modified medium to diminish production of pigmented compounds.
 6. Viti C, Decorosi F, Mini A, Tatti E, Giovannetti L: **Involvement of the *osCA* gene in the sulphur starvation response and in Cr(VI) resistance in *Pseudomonas corrugata* 28.** *Microbiology* 2009, **155**:95-105.
This study used biolug plates PM 1-4 and PM 9-20 to characterize changes in phenotype expression owing to gene deletion. Found that mutant lost some abilities to process select organo-sulfur compounds.
 7. Bender H-CY KS, Hemme CL, Yang Z, He Z, He Q, Zhou J, Huang KH, Alm EJ, Hazen TC, Arkin AP, Wall JD: **Analysis of a ferric uptake regulator (Fur) mutant of *Desulfovibrio vulgaris* Hildenborough.** *Appl Environ Microbiol* 2007, **73**:5389-5400.
 8. Perkins A, Nicholson W: **Uncovering new metabolic capabilities of *Bacillus subtilis* using phenotype profiling of rifampin-resistant *rpoB* mutants.** *J Bacteriol* 2008, **190**:807-814.
 9. Atanasova L, Druzhinina I: **Global nutrient profiling by Phenotype MicroArrays: a tool complementing genomic and proteomic studies in conidial fungi.** *Biomed Biotechnol* 2010, **11**:151-168.
Modified method using PM arrays for fungi, comparing wild-type and mutant strains. Excellent use of heat maps for data visualization.
 10. von Eiff C, McNamara P, Becker K, Bates D, Lei X-H, Ziman M, bochner B, Peters G, Proctor RA: **Phenotype Microarray profiling of *Staphylococcus aureus* menD and hemB mutants with the small-colony-variant phenotype.** *J Bacteriol* 2006, **188**:687-693.
 11. Armitano J, Baraquet C, Michotey V, Mejean V, Jourlin-Castellie C: **The chemical-in-mwell: a high-throughput technique for identifying solutes eliciting a chemotactic response in motile bacteria.** *Res Microbiol* 2011, **162**:934-938.
Utilized the compounds in the PM plates to inoculate a square petri dish with 96 agar wells and demonstrated chemotactic response of *Shewanella* spp to various compounds.
 12. Line J, Hielt K, Guard-Bouldin J, Seal B: **Differential carbon source utilization by *Campylobacter jejuni* 11168 in response to growth temperature variation.** *J Microbiol Methods* 2010, **80**:198-202.
Food safety application, compared growth of this strain at two temperatures representing the body temperature of chickens and humans. Used standard omnilog software for visualization.
 13. Decorosi F, Santopolo L, Mora D, Viti C, Giovannetti L: **The improvement of a phenotype microarray protocol for the chemical sensitivity analysis of *Streptococcus thermophilus*.** *J Microbiol Methods* 2011, **86**:258-261.
Describes technique modifications needed to complete assay because pH changes in the media affected dye coloration. Assayed antibiotic resistance changes in strains isolated from yogurt and pasteurized milk.
 14. Stolyar S, He Q, Joachimiak M, He Z, Yang Z, Borglin S, Joyner D, Huang K, Alm E, Hazen TC *et al.*: **Response of *Desulfovibrio vulgaris* to alkaline stress.** *J Bacteriol* 2007, **189**:8944-8952.
 15. Bochner B, Gomez V, Ziman M, Yang S, Brown S: **Phenotype MicroArray profiling of *Zymomonas mobilis* ZM4.** *Appl Biochem Biotechnol* 2010, **161**:116-123.
Classic use of PM for phenotype screening for this ethanol-producing organism. Found high ethanol tolerance and broad pH range for ethanol production.
 16. Rodrigues JLM, Serres MH, Tiedje JM: **Large scale comparative phenotypic and genomic analyses reveal ecological preferences of *Shewanella* species and identify metabolic pathways conserved at genus level.** *Appl Environ Microbiol* 2011. AEM.00097-00011.
 17. Bhupathiraju V, Hernandez M, Landfear D, Alvarez-Cohen L: **Application of a tetrazolium dye as an indicator of viability in anaerobic bacteria.** *J Microbiol Methods* 1999, **37**:231-243.
 18. Franks AE, Nevin KP, Glaven RH, Lovley DR: **Microtoming coupled to microarray analysis to evaluate the spatial metabolic status of *Geobacter sulfurreducens* biofilms.** *ISME J* 2010, **4**:509-519.
 19. Berridge M, Herst P, Tan A: **Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction.** *Biotechnol Annu Rev* 2005, **11**:127-152.
 20. Tachon S, Michelon D, Chambellon E, Cantonnet M, Mezange C, Henno L, Cachon R, Yvon M: **Experimental conditions affect the site of tetrazolium violet reduction in the electron transport chain of *Lactococcus lactis*.** *Microbiology* 2009, **155**:2941-2948.
 21. Zhou JZ, He Q, Hemme CL, Mukhopadhyay A, Hillesland K, Zhou AF, He ZL, Van Nostrand JD, Hazen TC, Stahl DA *et al.*: **How sulphate-reducing microorganisms cope with stress: lessons from systems biology.** *Nat Rev Microbiol* 2011, **9**:452-466.
 22. Oberhardt MG, Bielecka A, Regenhardt D, Timmis KN, Papin JA, Martins dos Santos VAP: **Genome-scale reconstruction and analysis of the *Pseudomonas putida* KT2440 metabolic network facilitates applications in biotechnology.** *PLoS Comput Biol* 2008, **4**:1-18.
Used selected plates from the omnilog array to validate metabolic model. Discussion of validity of dye usage as an assay for growth vs. assay for metabolism.
 23. de Vicente I, Amores V, Guerrero F, Cruz-Pizarro L: **Contrasting factors controlling microbial respiratory activity in the sediment of two adjacent Mediterranean wetlands.** *Naturwissenschaften* 2010, **97**:627-635.
 24. Joyner D, Fortney JL, Chakraborty R, Hazen TC: **Adaptation of the *Biolog* Phenotype MicroArray™ Technology to Profile the Obligate Anaerobe *Geobacter metallireducens*.** San Diego, CA: American Society for Microbiology; 2010 <http://escholarship.org/uc/item/3wr7t4cp>.
 25. Curtis TP, Sloan WT, Scannell JW: **Estimating prokaryotic diversity and its limits.** *Proc Natl Acad Sci USA* 2002, **99**:10494-10499.
 26. Bochner B: **Global phenotypic characterization of bacteria.** *FEMS Microbiol Rev* 2009, **33**:191-205.
 27. Bochner B, Giovannetti L, Viti C: **Important discoveries from analysing bacterial phenotypes.** *Mol Microbiol* 2008, **70**:274-280.
 28. Euzéby JP: *List of Prokaryotic Names with Standing in Nomenclature.* 2011 <http://www.bacterio.net>.
 29. Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu M, Tindall BJ *et al.*: **A phylogeny-driven genomic encyclopaedia of Bacteria and Archaea.** *Nature* 2009, **462**:1056-1060.

30. Vartoukian SR, Palmer RM, Wade WG: **Strategies for culture of 'unculturable' bacteria.** *FEMS Microbiol Lett* 2010, **309**:1-7.
31. Khudyakov J, D'haeseleer P, Borglin SE, DeAngelis K, Woo H, Lindquist E, Hazen TC, Simmons B, Thelen M: **Global transcriptome response to ionic liquid by a tropical rain forest soil bacterium, *Enterobacter lignolyticus* SCF1.** *PNAS* 2011, submitted.
32. DeAngelis KM, D'Haeseleer P, Chivian D, Fortney JL, Khudyakov J, Simmons B, Woo H, Arkin AP, Davenport KW, Goodwin L *et al.*: **Complete genome sequence of *Enterobacter lignolyticus* SCF1.** *SIGS* 2011, **5**:69-85.
33. Tremaroli V, Workentine ML, Weljie AM, Vogel HJ, Ceri H, Viti C, Tatti E, Zhang P, Hynes AP, Turner RJ *et al.*: **Metabolomic investigation of the bacterial response to a metal challenge.** *Appl Environ Microbiol* 2009, **75**:719-728.
34. Mukhopadhyay A, He Z, Alm EJ, Arkin AP, Baidoo EE, Borglin SC, Chen W, Hazen TC, He Q, Holman H-Y *et al.*: **Salt stress in *Desulfovibrio vulgaris* Hildenborough: an integrated genomics approach.** *J Bacteriol* 2006, **188**:4068-4078.
35. Zavrel M, Bross D, Funke M, Buchs J, Spiess AC: **High-throughput screening for ionic liquids dissolving (ligno-) cellulose.** *Bioresour Technol* 2009, **100**:2580-2587.
36. Hazen TC, Dubinsky EA, DeSantis TZ, Andersen GL, Piceno YM, Singh N, Jansson JK, Probst A, Borglin SE, Fortney JL *et al.*: **Deep-sea oil plume enriches indigenous oil-degrading bacteria.** *Science* 2010, **330**:204-208.
37. Pham TP, Cho CW, Yun YS: **Environmental fate and toxicity of ionic liquids: a review.** *Water Res* 2010, **44**:352-372.
38. Nicolaou SA, Gaida SM, Papoutsakis ET: **A comparative view of metabolite and substrate stress and tolerance in microbial bioprocessing: from biofuels and chemicals, to biocatalysis and bioremediation.** *Metab Eng* 2010, **12**:307-331.
39. Klimek B, Niklinska M: **Zinc and copper toxicity to soil bacteria and fungi from zinc polluted and unpolluted soils: a comparative study with different types of Biolog plates.** *Bull Environ Contam Toxicol* 2007, **78**:112-117.
40. Lu Z, Deng Y, Van Nostrand JD, He Z, Voordeckers J, Zhou A, Lee Y-J, Mason OU, Dubinsky EA, Chavarria KL *et al.*: **Microbial gene functions enriched in the Deepwater Horizon deep-sea oil plume.** *ISME J* 2011 doi: 10.1038/ismej.2011.91 2011.
41. Blomberg A: **Measuring growth rate in high-throughput growth phenotyping.** *Curr Opin Biotechnol* 2011, **22**:94-102.
42. Jasnos L, Sliwa P, Korona R: **Resolution and repeatability of phenotypic assays by automated growth curve analysis in yeast and bacteria.** *Anal Biochem* 2005, **344**:138-140.
43. Garland J, Mills A, Young J: **Relative effectiveness of kinetic analysis vs single point readings for classifying environmental samples based on community-level physiological profiles.** *CLPP* 2001, **33**:1059-1066.
44. Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP: **Summaries of Affymetrix GeneChip probe level data.** *Nucleic Acids Res* 2003, **31**:e15.
45. Saeed A, Bhagabati N, Braisted J, Liang W, Sharov V, Howe E, Li J, Thiagarajan M, White J, Quackenbush J: **TM4 microarray software suite.** *Methods Enzymol* 2006, **411**:134-193.
46. Dixon SJ, Heinrich N, Holmboe M, Schaefer ML, Reed RR, Trevejo J, Brereton RG: **Use of cluster separation indices and the influence of outliers: application of two new separation indices, the modified silhouette index and the overlap coefficient to simulated data and mouse urine metabolomic profiles.** *J Chemometrics* 2009, **23**:19-31.
47. Ripplinger J, Abdo Z, Sullivan J: **Effects of parameter estimation on maximum-likelihood bootstrap analysis.** *Mol Phylogenet Evol* 2010, **56**:642-648.
48. Weber K, Grove J, Gehder M, Anderson W, Legge R: **Data transformations in the analysis of community-level substrate utilization data from microplates.** *J Microbiol Methods* 2007, **69**:461-469.
49. Sturino J, Zorych I, Mallick B, Polusaeva K, Chang YY, Carroll R, Bliznyuk N: **Statistical methods for comparative phenomics using high-throughput phenotype microarrays.** *Int J Biostat* 2010, **6**:1-21.
50. Mondini C, Insam H: **Community level physiological profiling as a tool to evaluate compost maturity: a kinetic approach.** *Eur J Soil Biol* 2003, **39**:141-148.
51. DeNittis M, Zannoni B, Minati J, Gorra R, Ambrosoli R: **Modelling Biolog profiles' evolution for yeast growth monitoring in alcoholic fermentation.** *Lett Appl Microbiol* 2011, **52**:96-103.
52. Fodor IK, Holtz-Morris AE, McCutchen-Maloney SL: *Improving current microbial pathway models by statistical modeling of phenotype array experiments; In 2006 IEEE International Workshop on Genomic Signal Processing and Statistics: 2006:37-38.*
53. Fodor IK, Holtz-Morris AE, McCutchen-Maloney SL: *Growth Curve Models for the Analysis of Phenotype Arrays for a Systems Biology Overview of Yersinia pestis.* Medium: ED; Size: PDF-file: 9 pages; size: 0.7 Mbytes p, 2005.
54. Zwietering MH, Jongenburger I, Rombouts FM, VAN'T Riet K: **Modeling of the bacterial growth curve.** *Appl Environ Microbiol* 1990, **56**:1875-1881.

Older publication date, but a thorough comparison of different mathematical models to describe bacterial growth curves.