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Adaptation of the Biolog Phenotype MicroArray™ technology to profile the obligate anaerobe *Geobacter metallireducens*.

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http://vimss.lbl.gov/

Abstract

The Biolog OmniLog® Phenotype MicroArray (PM) plate technology was successfully adapted to generate a select phenotypic profile of the strict anaerobe *Geobacter metallireducens (G.m.)*. The profile generated for *G.m.* provides insight into the chemical sensitivity of the organism as well as some of its metabolic capabilities when grown with a basal medium containing acetate and Fe(III).

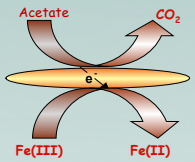
The PM technology was developed for aerobic organisms. The reduction of a tetrazolium dye by the test organism represents metabolic activity on the array which is detected and measured by the OmniLog® system. We have previously adapted the technology for the anaerobic sulfate reducing bacterium *Desulfovibrio vulgaris*. In this work, we have taken the technology a step further by adapting it for the iron reducing obligate anaerobe *Geobacter metallireducens*.

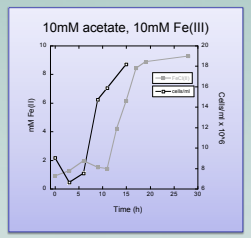
In an osmotic stress microarray it was determined that the organism has higher sensitivity to impermeable solutes 3-6% KCl and 2-5% NaNO3 that result in osmotic stress by osmosis to the cell than to permeable non-ionic solutes represented by 5-20% ethylene glycol and 2-3% urea.

The osmotic stress microarray also includes an array of osmoprotectants and precursor molecules that were screened to identify substrates that would provide osmotic protection to NaCl stress. None of the substrates tested conferred resistance to elevated concentrations of salt. Verification studies in which *G.m.* was grown in defined medium amended with 100mM NaCl (MIC) and the common osmoprotectants betaine, glycine and proline supported the PM findings. Further verification was done by analysis of transcriptomic profiles of *G.m.* grown under 100mM NaCl stress that revealed up-regulation of genes related to degradation rather than accumulation of the above-mentioned osmoprotectants. The phenotypic profile, supported by additional analysis indicates that the accumulation of these osmoprotectants as a response to salt stress does not occur in *G.m.* and response to stress must occur by other mechanisms.

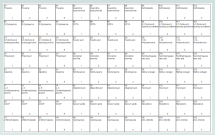
The Phenotype MicroArray technology can be reliably used as a rapid screening tool for characterization in anaerobic microbial ecology.

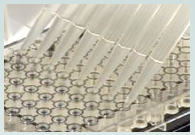
G.m. metabolism

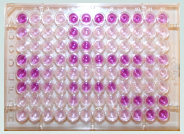




Method



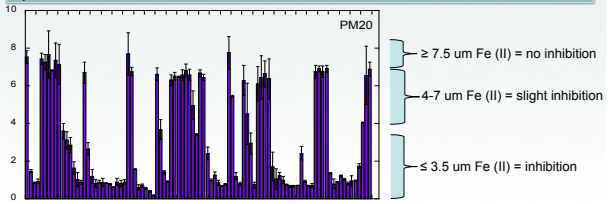




Ferrozine Assay for measurement of Fe(II)

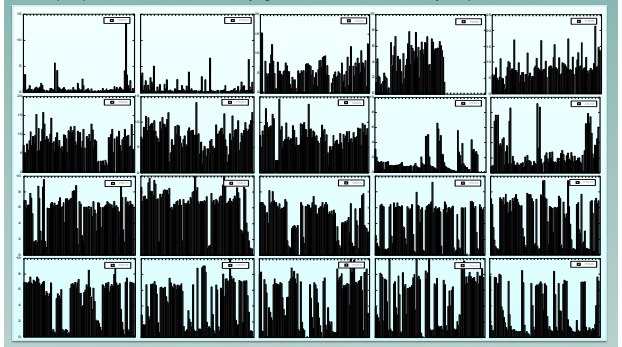
Reference: Stookey, L. L. 1970. Ferrozine--A New Spectrophotometric Reagent for Iron. *Analytical Chemistry* 42:779-781. (modified method for 96 well format)

1) 90ul 1N HCl + 1ul sample + 250ul ferrozine soln
2) Incubate 15min
3) Read at 562nm

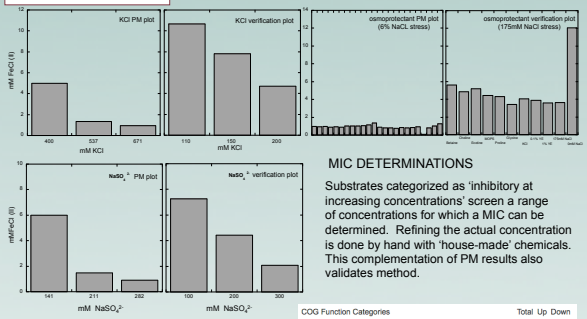


Phenotype MicroArray of *Geobacter metallireducens*

The complete Phenotype MicroArray consists of 20 panels which test for metabolism of carbon, nitrogen, sulfur, phosphate substrates as well as varying concentrations of 240 inhibitory compounds.



Method Validation



MIC DETERMINATIONS

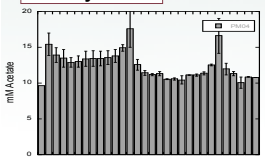
Substrates categorized as 'inhibitory at increasing concentrations' screen a range of concentrations for which a MIC can be determined. Refining the actual concentration is done by hand with 'house-made' chemicals. This complementation of PM results also validates method.

Transcriptomic Results

The osmotic stress panel highlighted an interesting phenotype for salt stress response. We investigated this further by determining the MIC for the ionic salts NaCl and KCl and testing the effect of a variety of osmoprotectants at these MIC concentrations. After verifying that no phenotypic osmoprotection was seen, the genetic response was studied under salt stress. We observed that under salt stress cell motility, and signal transduction genes are down regulated. Upregulated genes include those for amino acid transport and metabolism, cell wall/membrane / envelope biogenesis, coenzyme transport and lipid transport

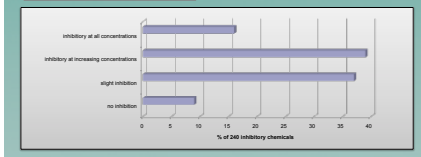
COG Function Categories	Total	Up	Down
Amino acid transport and metabolism	157	42	7
Carbohydrate transport and metabolism	94	16	11
Cell cycle control, cell division, chromosome partitioning	25	4	2
Cell motility	119	0	52
Cell wall/membrane/envelope biogenesis	190	32	5
Chromatin structure and dynamics	1	0	0
Coenzyme transport and metabolism	129	31	6
Defense mechanisms	34	2	0
Energy production and conversion	279	29	34
Function unknown	195	32	22
General function prediction only	289	29	31
Inorganic ion transport and metabolism	108	9	14
Intracellular trafficking, secretion, and vesicular transport	89	16	17
Lipid transport and metabolism	134	22	5
Nucleotide transport and metabolism	58	26	3
Posttranslational modification, protein turnover, chaperones	108	21	17
Replication, recombination and repair	162	12	2
Secondary metabolites biosynthesis, transport and catabolism	54	9	4
Signal transduction mechanisms	191	5	43
Transcription	124	18	10
Translation, ribosomal structure and biogenesis	159	84	2

IC Analysis



Measurement of acetate utilization is an alternative assay to measure metabolism of *G.m.* on the substrate of interest. This may be needed for verification of a PM result since abiotic reduction or interference of ferrozine measurement of iron by some of the compounds may occur. IC analysis of acetate concentration was done for the sulfur panel to determine if any of the compounds tested could be used as electron acceptors with acetate as the donor. None of the compounds tested showed acetate utilization above background.

Summary of PM



240 inhibitory chemicals and toxic compounds were tested and the results classified into the following 4 categories.

No inhibition

Thiosalicylate	biofilm inhibitor, anti-capsule agent, chelator, prostaglandin syntetase inhibitor
5-Chloro-7-iodo-8-hydroxy-quinoline	chelator, lipophilic
Compound 48/80	cyclic AMP phosphodiesterase inhibitor
Myricetin	DNA & RNA synthesis, polymerase inhibitor
Hexammine cobalt (III) chloride	DNA synthesis
Novobiocin	DNA topoisomerase
Nordihydroguaiaretic acid	lipoxygenase, fungicide
Polymyxin B	membrane, cyclic peptide
Tetraethylthiuram disulfide	nucleic acid inhibitor, purine
Plumbagin	oxidizing agent
Phenyl-methylsulfonyl-fluoride (PMSF)	protease inhibitor, serine
Chlortetracycline, Perimepicycline	protein synthesis, 30S ribosomal subunit, tetracycline
Ferric chloride	toxic cation
Ceftriaxone, Cefoxitin, Cefamandole nafate	wall, cephalosporin
Amikacin, Carbenicillin, Moxalactam, Aztreonam	wall, lactam

Inhibitory at all concentrations tested

Semicarbazide hydrochloride	amine oxidase inhibitor, carcinogen
Orphenadrine, Pridinol	anti-cholinergic
Sanguinarine chloride	ATPase, Na+/K+ and Mg++
Acriflavine	DNA intercalator, inhibits RNA synthesis
Dichlofluanid, Tolylfluanid	fungicide, phenylsulphamide
D-Serine	inhibits 3PGA dehydrogenase (L-serine and pantothenate synthesis)
3-Amino-1,2,4-triazole	inhibits catalase, inhibits histidine synthesis
Dodine, Guanidine hydrochloride, Dodecyltrimethyl ammonium bromide, Lauryl sulfobetaine	membrane
Patulin	microtubulin polymerization inhibitor, antifungal
Captan	multisite, carbamate, fungicide
Tinidazole, Ornidazole, 2- Nitroimidazole	nitro compound, oxidizing agent
6-Mercaptopurine monohydrate	nucleic acid analog, purine
Chlorodinitrobenzene	oxidizes sulfhydryls, depletes glutathione
2-Hydroxy-1,4-Naphthoquinone	oxidizing agent
Lincomycin, Tylosin tartrate, Thiamphenicol, Fusidic acid,	protein synthesis
INT, CCCP, FCCP, 3,5-Dinitrobenzoic acid, Tetrazolium violet, Thioridazine	respiration
Oxycarboxin	respiratory enzymes, carboxamide, fungicide
Sodium nitrite, Sodium selenite , Sodium bromate, Sodium tungstate, Sodium periodate	toxic anion
Glycine hydroxamate	tRNA synthetase

Conclusions of PM array

-The Phenotype MicroArray has been adapted to profile an iron-reducing, low biomass, obligate anaerobe. Over 1,000 aerobes have been profiled by the Biolog OmniLog® PM. We have previously adapted the technology to profile a sulfate reducing bacterium. This is the first report on an obligate iron-reducing anaerobe.

- PM results presented here highlighted interesting responses to osmotic stress which we investigated further. *G.m.* shows higher sensitivity to ionic permeable solutes than to non-ionic permeable solutes. Osmoprotection to osmotic stress does not appear to be a mode of stress response by *G.m.* to osmolytes tested. This is validated by complementary verification assays as well as by transcriptomic data.

-This screen is helpful in identifying compounds that can stimulate as well as inhibit growth. Several metabolic as well as inhibitory compounds were highlighted as interesting candidates for further investigation. These results will be helpful in molecular biological applications.

- Histidine containing dipeptides were inhibitory.

-15% of the chemicals tested resulted in no growth. These chemicals were classified into the following categories: antimicrobials, antiseptic, fungicide, antibiotic, herbicide, anti parasitic/protozoan, chemical inhibitors of basic metabolic functioning ie) respiration.

- 9% of the inhibitory compounds tested resulted in no inhibition of G.m.. This list includes antibiotic/ antimicrobial/fungicide compounds.

- Future studies will utilize this method to generate phenotypic profiles of other dominant anaerobic iron-reducers in the environment to lend an understanding of the microbial ecology of this group of organisms.

ACKNOWLEDGEMENTS

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