Biolog, Inc.
21124 Cabot Blvd.
Hayward, California 94545
510-785-2564 Telephone
800-284-4949 Orders
510-782-4639 Fax
www.biolog.com

Phenotype MicroArray Services

BIOLOG

Customer:	Lisa Morici				
# Strains:	1, *P. put.*; 1, mix culture	**# conditions:**	1	**Temperature:**	25C
Profile Type:	Full Profile	**plates/profile:**	20	**#pairwise comparisons:**	1

Dear Dr. Morici,

Thank you for using Phenotype MicroArray™ (PM) Services. This confirms the completion of your PM Services and details the results. We have completed duplicate runs of each of the strains/conditions and created **pairwise** comparisons according to your instructions. Please use this letter as a guide to walk through the attached reports.

Attached you will find OmniLog® V. 1.5 Comparison Module reports (MS Word documents) of each comparison with images of the profiles.

a. Reproducibility report containing correlation plots of the independent runs. Reproducibility analysis indicates the number of wells where the difference of average height between duplicate runs is above a threshold value, which is indicated in the report. Average height is the area under the curve divided by number of reads. Pass/fail is determined by the number of such wells above a threshold value (usually 12 wells).
b. Run1 and Run2 images. Test in green and reference in red.
c. An image with consensus calls. "average height" is the sum of the reads divided by the number of reads. Boxed wells exceed the "average height" threshold in both of the two independent experiments. This means that the absolute value of the arithmetic difference (test minus reference) is above threshold. Positive differences may be gained phenotypes or resistance of the test strain relative to the reference strain. Negative differences may be lost phenotypes or sensitivity of the test strain relative to the reference strain.
d. A report of wells above threshold.
e. Please note that the filename is usually built using the format "test versus reference.doc".

Find information on PM Technology at: http://www.biolog.com/phenoMicro.html
Find information on PM Services at: http://www.biolog.com/PM_Services.html
Find PM maps at the following URL: http://www.biolog.com/PM_Maps.html
Please review our Frequently Asked Questions: http://www.biolog.com/PM_FAQ.html

PsppSIDx5842xMixCulture versus PputSIDx5841xPputida

All plates passed reproducibility analysis at 24 hours of incubation. The mix culture grew more slowly in some conditions (eg. PM5) and was less reproducible at timepoints later than 24 hours.

1) Carbon utilization (PM1,2): Positive differences were observed for most categories of carbon sources except alcohols. Positive differences for amino acids were limited to dipeptides. Negative differences were observed for L-amino acids and carboxylic acids.
2) Nitrogen utilization (PM3, 6-8): Positive differences were observed for many dipeptides and a few other nitrogen sources. Negative differences were seen across all categories of nitrogen sources including most amino-acids.
3) Phosphorus and Sulfur (PM4): Positive differences include 2',3' cyclic nucleotides. Negative differences include a variety of organic and inorganic phosphorus and sulfur sources.
4) Nutrient Stimulation (PM5): The mix culture did not come up until after 24 hours for the most part. Stimulation of the mix culture was observed for oxalacetic acid (PM5, G10) and perhaps a few others.
5) Osmolarity and pH (PM9, 10): Negative differences were observed for benzoate and nitrate (PM9, G5-6; H4-6). A negative difference was observed for pH=4.5 (PM10, A3). Positive differences were observed for growth at pH=9.5 in the presence of L-norleucine (PM10, G2), L-histidine (PM10, E9), and L-phenylalanine (PM10, F2).
6) Chemical sensitivity (PM11-20): Positive differences include chelators, membrane disruptors, anti-cholinergics, respiration inhibitors, toxic ions, and others. Negative differences include capsule inhibitors, inhibitors of nucleic acid metabolism, oxidizing agents, nucleotide analogs, various antibiotics, and ionophores.

We hope that this information is useful to you in your studies. As a next step, we recommend that you independently verify any phenotypes that you consider important. Please let us know if you follow-up on these phenotypic leads. It will be very useful to us. We are more than happy to consult with you on methods for the validation of these observations. Please contact me to discuss this data over the phone (510-670-3370).

Biolog can provide you with the fully automated OmniLog system for PM testing in your own laboratory. Please contact sales@biolog.com or call 510-785-2564 if you would like more information. Our sales staff will follow up with you regarding the use of PM systems in your research efforts.

Sincerely,
Michael Ziman, Ph.D.

Michael Ziman

Director, PM Services

PM Services
Biolog, Inc.
21124 Cabot Blvd.
Hayward, CA 94545

T: 510-670-3370
F: 510-782-4639
E: pmservices@biolog.com
W: http://www.biolog.com/PM_Services.html

Sewper Rx Advantages versus *P. putida*: Increased usage of carbon sources!

Phenotypes Gained:				
PM17A	A08	61	b-Chloro-L-alanine	aa analog, alanine, aminotransferase inhibitor
PM20B	A09	82	Benserazide	aa metabolism, aromatic amino acid decarboxylase inhibitor
PM18C	F02,F03	114	Semicarbazide hydrochloride	amine oxidase inhibitor, carcinogen
PM20B	B03	67	Orphenadrine	anti-cholinergic
PM01	H01	125	Gly-Pro	C-Source, amino acid
PM01	F01	104	Gly-Asp	C-Source, amino acid
PM01	G06	92	Ala-Gly	C-Source, amino acid
PM01	G01	81	Gly-Glu	C-Source, amino acid
PM01	C01	186	D-Glucose-6-Phosphate	C-Source, carbohydrate
PM01	E04	175	D-Fructose-6-Phosphate	C-Source, carbohydrate
PM01	B07	147	D,L-a-Glycerol Phosphate	C-Source, carbohydrate
PM01	E03	144	D-Glucose-1-Phosphate	C-Source, carbohydrate
PM01	F12	131	Inosine	C-Source, carbohydrate
PM01	A06	128	D-Galactose	C-Source, carbohydrate
PM01	E12	127	Adenosine	C-Source, carbohydrate
PM01	D09	122	a-D-Lactose	C-Source, carbohydrate
PM01	B08	120	D-Xylose	C-Source, carbohydrate
PM01	G08	120	N-Acetyl-D-Mannosamine	C-Source, carbohydrate
PM01	B11	111	D-Mannitol	C-Source, carbohydrate
PM01	C04	111	D-Ribose	C-Source, carbohydrate
PM01	C10	108	Maltose	C-Source, carbohydrate
PM01	A02	107	L-Arabinose	C-Source, carbohydrate
PM01	C11	106	D-Melibiose	C-Source, carbohydrate
PM01	D11	105	Sucrose	C-Source, carbohydrate
PM02A	E05	104	D-Glucosamine	C-Source, carbohydrate
PM01	A11	102	D-Mannose	C-Source, carbohydrate
PM01	C12	100	Thymidine	C-Source, carbohydrate
PM01	E09	99	Adonitol	C-Source, carbohydrate
PM01	E11	97	2`-Deoxyadenosine	C-Source, carbohydrate
PM01	C06	94	L-Rhamnose	C-Source, carbohydrate
PM01	A10	93	D-Trehalose	C-Source, carbohydrate
PM01	E10	93	Maltotriose	C-Source, carbohydrate
PM01	B03	92	Glycerol	C-Source, carbohydrate
PM02A	B06	91	D-Arabitol	C-Source, carbohydrate
PM01	F11	90	D-Cellobiose	C-Source, carbohydrate
PM02A	C07	87	b-Methyl-D-Galactoside	C-Source, carbohydrate
PM01	A03	83	N-Acetyl-D-Glucosamine	C-Source, carbohydrate
PM02A	B08	83	Arbutin	C-Source, carbohydrate
PM02A	D01	83	D-Raffinose	C-Source, carbohydrate

Sewper Rx Advantages: resistance to chelators and detergents

PM01	E08	82	b-Methyl-D-Glucoside	C-Source, carbohydrate
PM01	A12	80	Dulcitol	C-Source, carbohydrate
PM01	D12	80	Uridine	C-Source, carbohydrate
PM02A	B12	79	3-0-b-D-Galactopyranosyl-D-Arabinose	C-Source, carbohydrate
PM01	D08	77	a-Methyl-D-Galactoside	C-Source, carbohydrate
PM02A	D02	77	Salicin	C-Source, carbohydrate
PM02A	C01	75	Gentiobiose	C-Source, carbohydrate
PM01	F03	72	m-Inositol	C-Source, carbohydrate
PM01	B02	62	D-Sorbitol	C-Source, carbohydrate
PM02A	C12	62	Palatinose	C-Source, carbohydrate
PM01	H10	149	D-Galacturonic Acid	C-Source, carboxylic acid
PM01	H08	130	Pyruvic Acid	C-Source, carboxylic acid
PM01	B05	125	D-Glucuronic Acid	C-Source, carboxylic acid
PM01	C02	113	D-Galactonic Acid-g-Lactone	C-Source, carboxylic acid
PM01	H02	102	p-Hydroxyphenyl Acetic Acid	C-Source, carboxylic acid
PM02A	B02	88	N-Acetyl-Neuraminic Acid	C-Source, carboxylic acid
PM01	B10	85	Formic Acid	C-Source, carboxylic acid
PM01	H03	75	m-Hydroxyphenyl Acetic Acid	C-Source, carboxylic acid
PM01	D03	71	D-Glucosaminic Acid	C-Source, carboxylic acid
PM01	G10	84	Methylpyruvate	C-Source, ester
PM02A	A06	104	Dextrin	C-Source, polymer
PM14A	H03,H04	142	EGTA	chelator, Ca++
PM15B	B05,B06,B07,B08	372	EDTA	chelator, hydrophilic
PM18C	A07,A08	241	Pyrophosphate	chelator, hydrophilic
PM17A	E06,E07,E08	356	Compound 48/80	cyclic AMP phosphodiesterase inhibitor
PM20B	D02,D03,D04	202	Proflavine	DNA intercalator, inhibits RNA synthesis
PM19	H04	102	Hexamminecobalt (III) Chloride	DNA synthesis
PM12B	F07,F08	139	Sulfathiazole	folate antagonist, PABA analog
PM17A	D05,D06,D07	166	Aminotriazole	inhibits catalase, inhibits histidine synthesis
PM11C	C07,C08	181	Colistin	membrane, cyclic peptide
PM19	H11,H12	136	Polymyxin B	membrane, cyclic peptide
PM12B	B12	88	Polymyxin B	membrane, cyclic peptide
PM17A	E02,E03,E04	132	Niaproof	membrane, detergent, anionic
PM19	B04	106	Methyltrioctylammonium chloride	membrane, detergent, cationic
PM15B	D07	85	Domiphen bromide	membrane, detergent, cationic, fungicide
PM19	G02,G03,G04	237	Lauryl sulfobetaine	membrane, detergent, zwitterionic
PM20B	A03	81	Amitriptyline	membrane, transport
PM03B	A11	138	L-Cysteine	N-Source, amino acid
PM03B	F01	66	N-Acetyl-D-Mannosamine	N-Source, other
PM03B	E08	65	D-Glucosamine	N-Source, other
PM03B	E11	61	N-Acetyl-D-Glucosamine	N-Source, other
PM08	H01	97	Gly-Gly-Ala	N-Source, peptide
PM08	A08	96	Asp-Ala	N-Source, peptide
PM08	A03	92	Ala-Asp	N-Source, peptide
PM08	D11	91	Thr-Asp	N-Source, peptide
PM08	D04	90	Pro-Ser	N-Source, peptide
PM03B	H01	87	Ala-Asp	N-Source, peptide

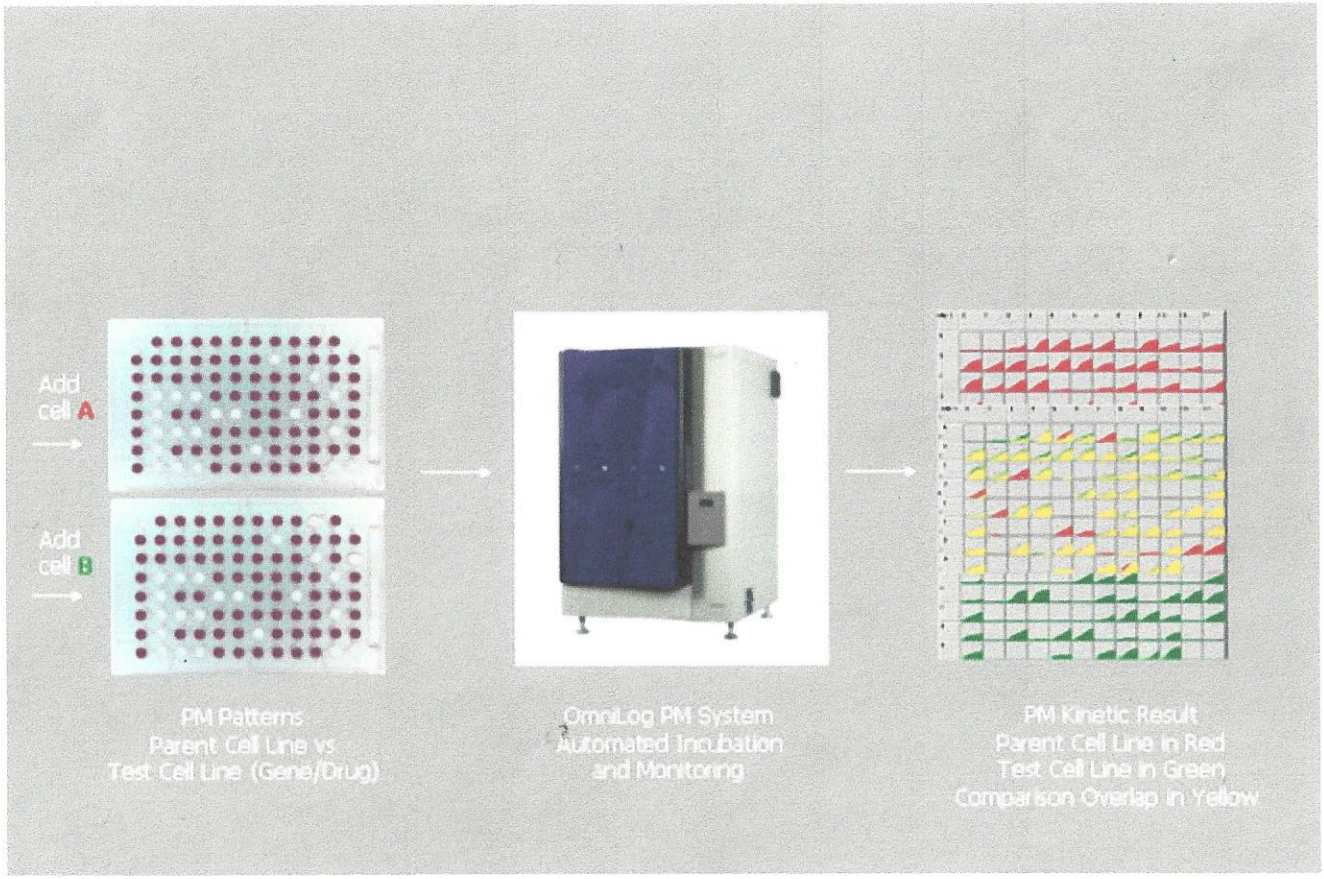
Sewper Rx Advantages: increased usage of dipeptides as N-sources

PM07	E07	87	Ser-Pro	N-Source, peptide
PM08	B01	85	Gly-Asp	N-Source, peptide
PM08	D08	83	Ser-Asp	N-Source, peptide
PM08	H03	82	Gly-Gly-Gly	N-Source, peptide
PM08	G11	81	Ala-Ala-Ala	N-Source, peptide
PM08	H04	80	Gly-Gly-Ile	N-Source, peptide
PM08	C12	79	Pro-Asn	N-Source, peptide
PM08	A10	78	Asp-Gly	N-Source, peptide
PM08	H06	78	Gly-Gly-Phe	N-Source, peptide
PM08	A09	77	Asp-Gln	N-Source, peptide
PM08	D06	77	Pro-Val	N-Source, peptide
PM07	F05	74	Thr-Pro	N-Source, peptide
PM07	C06	73	Met-Pro	N-Source, peptide
PM08	E12	73	Val-Pro	N-Source, peptide
PM08	D02	72	Pro-lle	N-Source, peptide
PM06	E11	70	Gly-Pro	N-Source, peptide
PM08	H11	70	Phe-Gly-Gly	N-Source, peptide
PM07	D05	69	Pro-Asp	N-Source, peptide
PM08	B10	68	Leu-Pro	N-Source, peptide
PM06	A12	67	Ala-Pro	N-Source, peptide
PM07	D04	67	Pro-Ala	N-Source, peptide
PM06	C10	66	Asp-Glu	N-Source, peptide
PM06	D01	66	Asp-Phe	N-Source, peptide
PM03B	H10	63	Gly-Glu	N-Source, peptide
PM08	C05	63	Phe-Asp	N-Source, peptide
PM08	H12	63	Tyr-Gly-Gly	N-Source, peptide
PM06	D09	62	Glu-Gly	N-Source, peptide
PM06	C11	59	Asp-Leu	N-Source, peptide
PM07	D01	59	Phe-Pro	N-Source, peptide
PM06	D08	58	Glu-Glu	N-Source, peptide
PM06	D11	58	Glu-Trp	N-Source, peptide
PM07	D11	58	Pro-Pro	N-Source, peptide
PM07	D09	57	Pro-Leu	N-Source, peptide
PM07	D06	55	Pro-Gln	N-Source, peptide
PM07	D10	53	Pro-Phe	N-Source, peptide
PM08	C11	53	Pro-Arg	N-Source, peptide
PM06	F10	52	His-Pro	N-Source, peptide
PM07	F08	51	Trp-Asp	N-Source, peptide
PM07	H05	51	Val-Asp	N-Source, peptide
PM12B	F09,F10,F11,F12	395	5-Fluoroorotic acid	nucleic acid analog, pyrimidine
PM04A	A11	62	Adenosine 2`,3`-Cyclic Monophosphate	P-Source, organic
PM04A	B11	56	Guanosine 2`,3`-Cyclic Monophosphate	P-Source, organic
PM04A	D11	54	Uridine 2`,3`-Cyclic Monophosphate	P-Source, organic
PM10	G02	92	pH 9.5 + L-Norleucine	pH, deaminase
PM10	E09	90	pH 9.5 + L-Histidine	pH, deaminase
PM10	F02	83	pH 9.5 + L-Phenylalanine	pH, deaminase
PM17A	D11	90	Chlorpromazine	phenothiazine, anti-cholinergic, anti-psychotic, sedative

Sewper Rx Advantages: resistance to toxic anions and cations

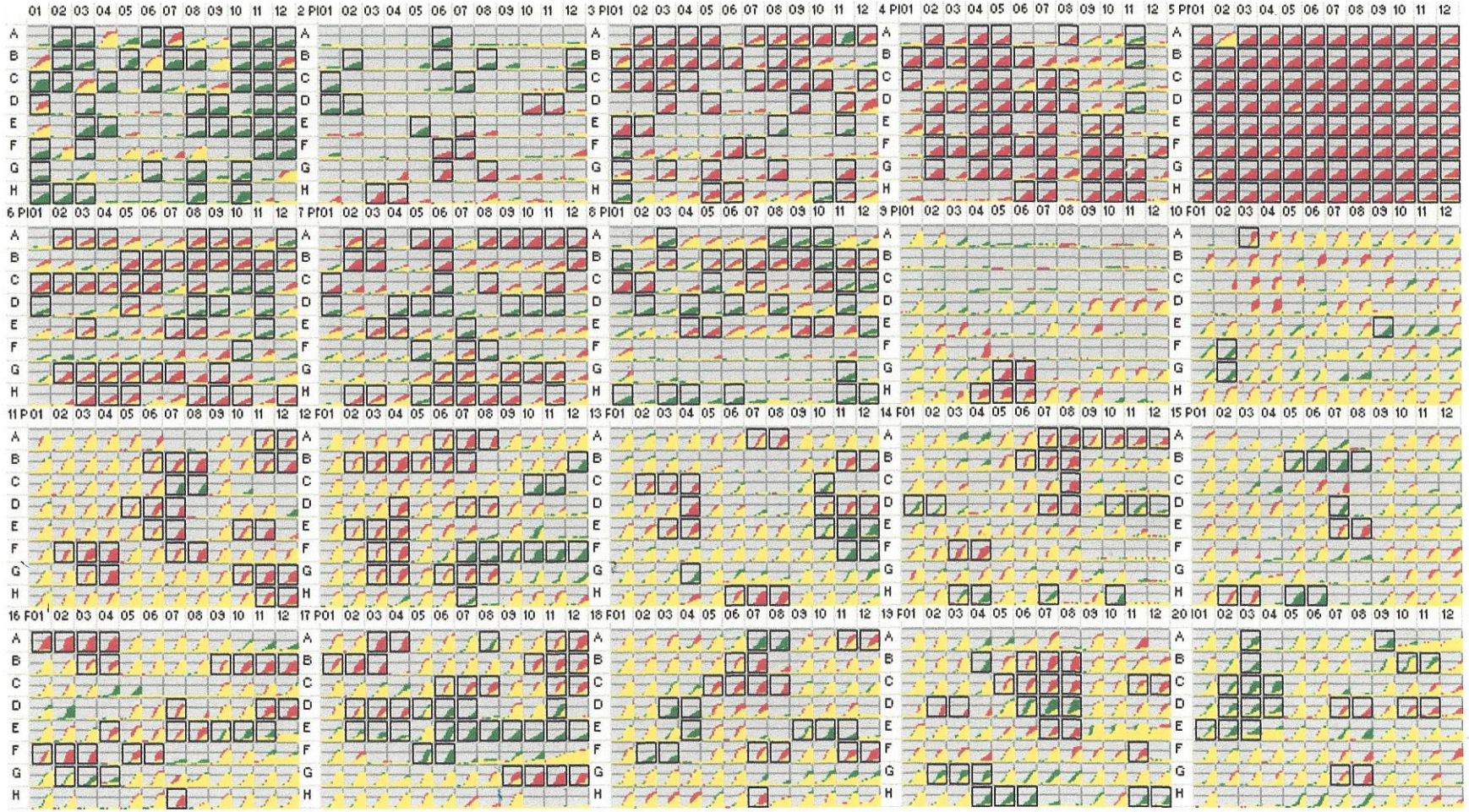
PM14A	H07	61	Promethazine	phenothiazine, anti-cholinergic, anti-psychotic, sedative
PM19	H05,H06	197	Thioglycerol	reducing agent, thiol, adenosyl methionine antagonist
PM19	D06,D07,D08	379	Iodonitro tetrazolium violet	respiration
PM13B	E10,E11,E12	273	Ruthenium red	respiration, mitochondrial Ca++ porter
PM20B	C02,C03,C04	275	Thioridazine	respiration, uncoupler
PM20B	E01,E02,E03	176	Crystal Violet	respiration, uncoupler
PM20B	B10,B11	123	Tetrazolium violet	respiration, uncoupler
PM15B	H05,H06	221	Hydroxyurea	ribonucleotide DP reductase inhibitor, antifolate (inhibits thymine and methionine synthesis)
PM16A	E09,E10,E11	195	Rifamycin SV	RNA polymerase
PM12B	H07	62	Rifampicin	RNA polymerase
PM18C	D03,D04	159	Sodium m-arsenite	toxic anion
PM18C	E04	96	Sodium metasilicate	toxic anion
PM13B	C10	72	Potassium chromate	toxic anion
PM17A	E09,E10,E11,E12	286	Sodium tungstate	toxic anion, molybdate analog
PM14A	H10	68	Sodium orthovanadate	toxic anion, PO4 analog
PM14A	D10,D11,D12	134	Sodium dichromate	toxic anion, SO4 analog
PM18C	E09,E10,E11	169	Antimony (III) chloride	toxic cation
PM16A	G02,G03,G04	147	Chromium chloride	toxic cation
PM13B	F11,F12	142	Thallium (I) acetate	toxic cation
PM14A	D01,D02	100	Cadmium chloride	toxic cation
PM13B	G04	68	Cobalt chloride	toxic cation
PM17A	F05,F06	207	D,L-Methionine hydroxamate	tRNA synthetase
PM12B	C10,C11	158	D,L-Serine hydroxamate	tRNA synthetase

Biolog Phenotype Array Technology



Sewper Rx Phenotype Arrays (PM1-20)

Consensus:
Pspp_SID_5842_MixCulture(green)
,versus
Pput_SID_5841_Pputida(red)



Sewper Rx Substrate Utilization

(not all-inclusive)

Carbon: See extensive list on previous slides. Lactose, etc.

Nitrogen: Ammonia (Nitrate, Nitrite and Urea mostly *P. putida* in first 24 hrs).

Phosphorus: Phosphoglycolic acid; glucose phosphate; glucosamine phophate (phosphate alone - mostly *P. putida*).

Sulfur: All *P. putida* - sulfate, thiosulfate, thiophosphate, butane sulfonic acid, hydroxyethane sulfonic acid; methane sulfonic acid.

Sewper Rx Tolerance Characteristics

pH range: 4.5 to 10; above 10 not tested. Bacterial growth inhibited at pH 4.0 and below.

Salt tolerance: Grew in 1-3% NaCl; inhibited by 4% NaCl

Potassium Chloride (used in fertilizers): Grew in 3 and 4% KCl; inhibited at 5% or greater.

Sodium sulfate (used in detergents): Grew in 2-5%. 5% highest tested.

Ethylene glycol (used in antifreeze): Grew in 5-20%; 20% highest tested.

Urea (used in fertilizers as source of N): Grew in 2-4%; inhibited by 5% or greater.

Sodium phosphate (food additive; emulsifier for processed cheeses): grew in 20-200 mM (pH 7); 200 highest conc tested.

Ammonium sulfate (fertilizer- lowers pH and provides source of N): grew in 10-100 mM (pH 8); 100 highest conc tested.