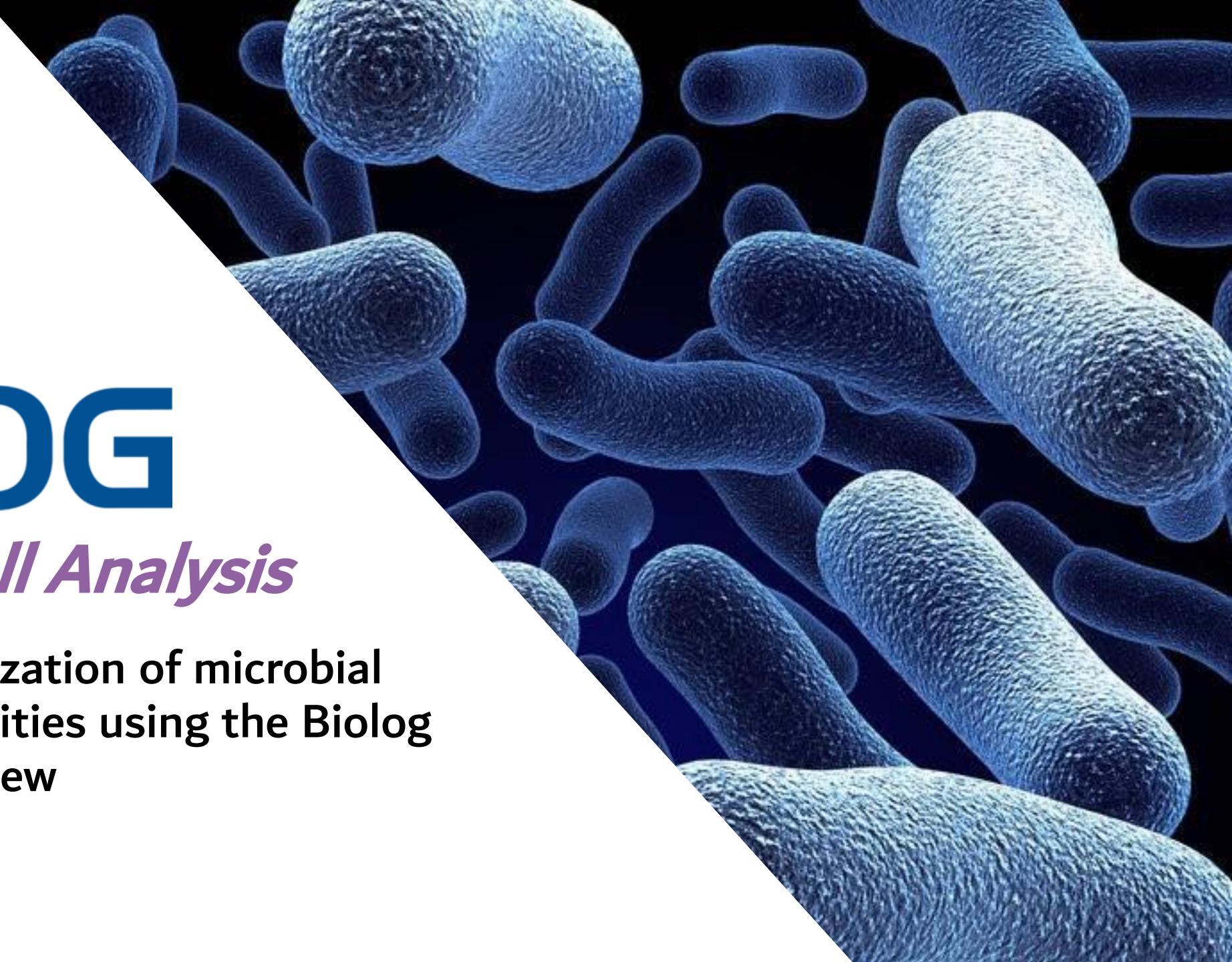




*A Leader in Cell Analysis*

Functional characterization of microbial isolates and communities using the Biolog technology: an overview

Enrico Tatti, PhD



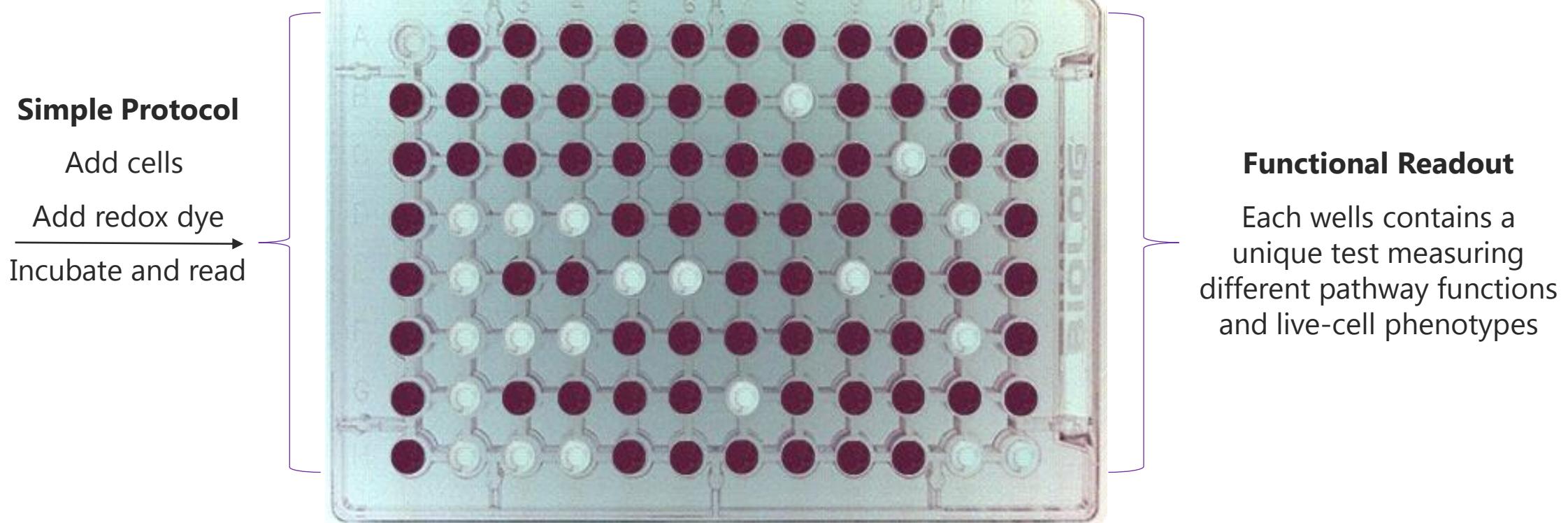
# Agenda

- Biolog Technology: how does it work?
- The beginning: Microbial ID
- The development of Phenotype MicroArray
- Microbial Communities Functional Analysis
- Beyond Microbes

# Exploiting the Phenotype: Microbial ID

# Redox chemistry measures live-cell metabolism

Microplate containing a negative control well and 95 different substrates

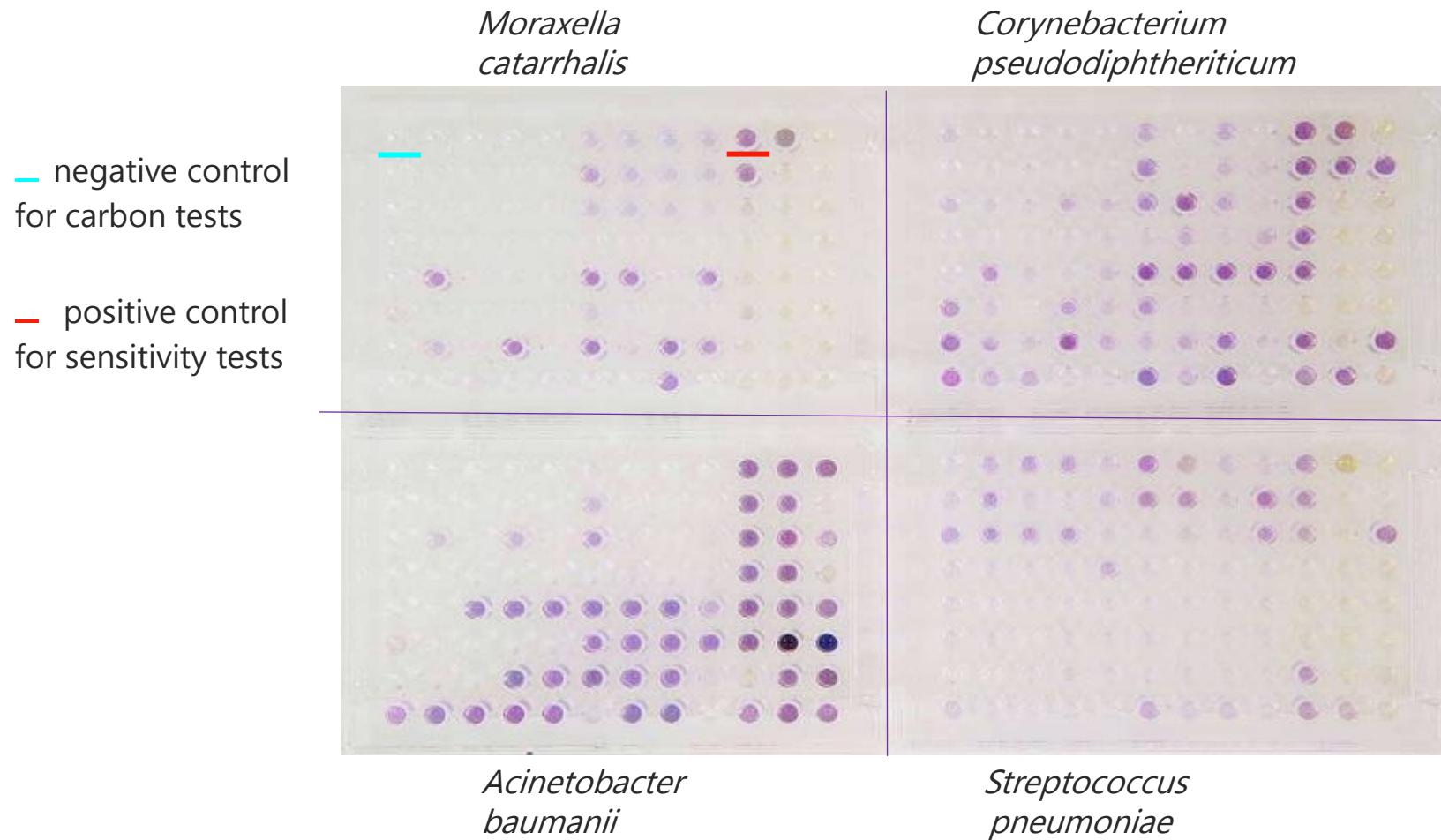


# L. E. Den Dooren de Jong - 1926



Den Dooren de Jong proved that microbes could be distinguished based on their utilization of different sets of carbon sources

# Different Microbes Gives Different Profiles



# Streamlined protocol



Isolate



Prepare

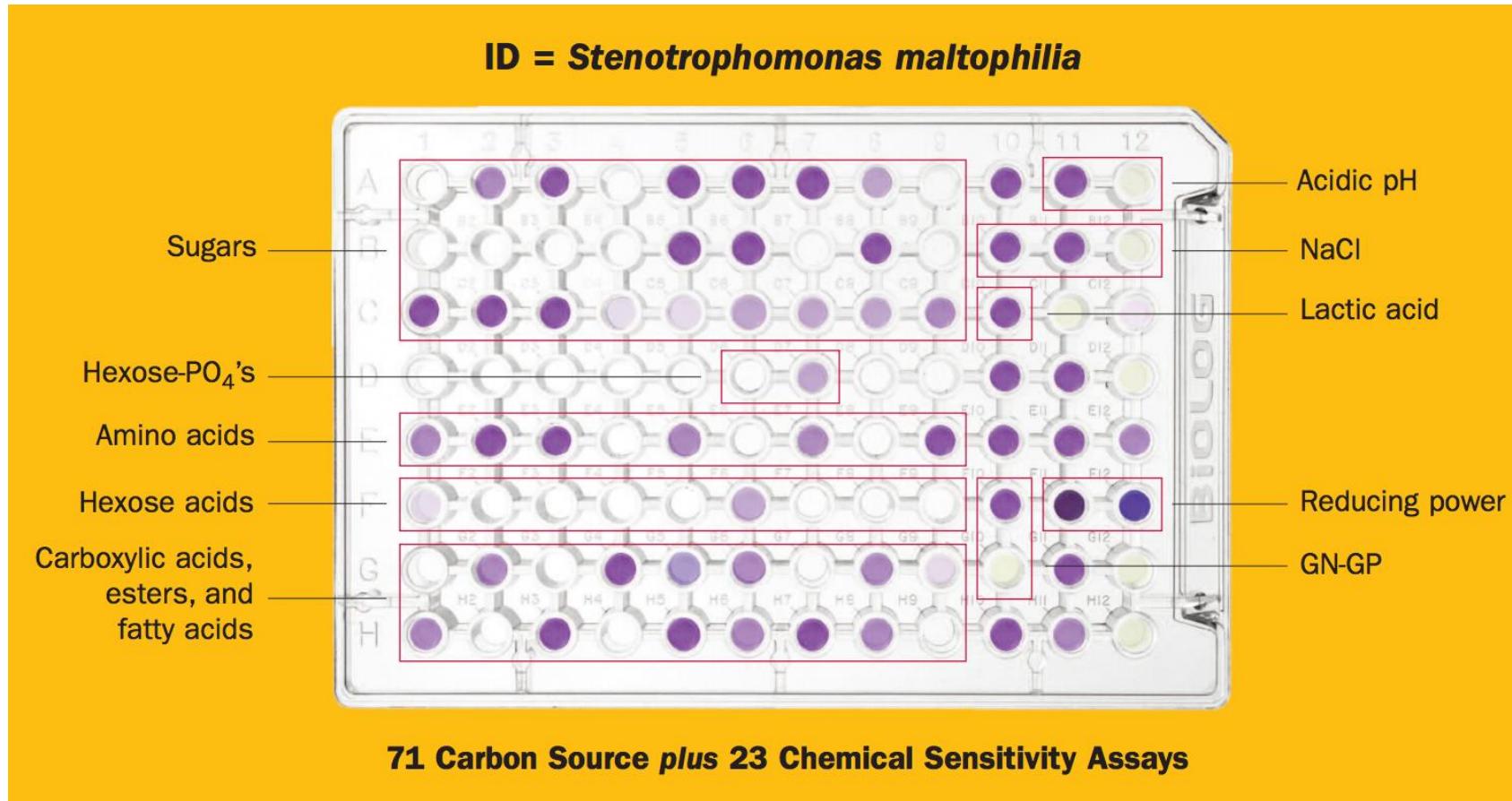


Inoculate



Incubate and Read

# Anatomy of a GEN III identification



# ID and Biological Information

View Details

Field	Value
Project	PQ
Plate Number	1
Plate Type	GEN III
Protocol	B
Strain Type	
Incubation Hours	22
Sample Number	<i>B. choshinensis</i>
Sample Name	Bioburden 2
IF Lot No.	20OCT221
Plate Lot No.	3412171
Media	
OD/Transmittance	
Technician	Federico
Room No.	
Comments	
Date	

Pos/Neg Graphic | Pos/Neg Numerical | ODs

	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	●	●	●	○
B	○	○	○	○	○	○	○	○	●	●	●	●
C	○	○	○	○	○	○	○	○	●	○	○	○
D	○	○	○	○	●	○	○	●	●	○	○	○
E	○	○	●	○	●	●	●	○	●	●	●	○
F	○	○	○	●	○	●	○	○	○	○	●	○
G	○	○	○	○	●	●	○	●	○	○	●	●
H	●	○	○	●	○	●	○	●	○	●	●	○

D12 Minocycline

Species ID: *Bacillus acidicola*

	SIM	DIST	Organism Type	Species
==>1	0.638	5.247	GP-Rod-SB	<i>Bacillus acidicola</i>
2	0.168	5.789	GP-Rod-SB	<i>Bacillus fortis</i>
3	0.137	5.968	GP-Rod-SB	<i>Bacillus farraginis</i>
4	0.056	6.745	GP-Rod-SB	<i>Bhargavaea ginsengi</i>

Compare Data To Other Species

X

View Selected Species

Compare To Other Species

Clear Species

Print Preview

Print

Close

High resolution QC of obtained results

The Biolog logo consists of a stylized grid of colored dots in shades of blue and purple, followed by the word "Biolog" in a bold, sans-serif font.

# Designed to ID common bugs and the weird stuff

## Comprehensive Database

- Biolog's Database covers
  - 1,568 aerobic taxa
  - 361 anaerobic taxa
  - 267 yeast species
  - 710 filamentous fungi species
- Users can develop custom ID databases

## Superior Chemistry

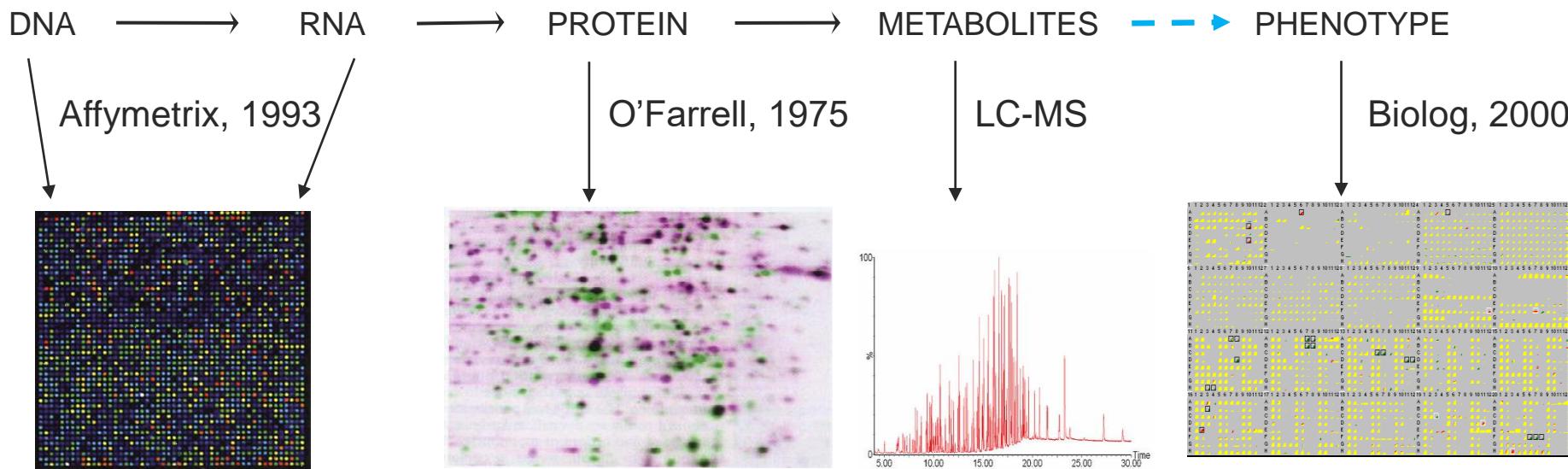
- Biolog incorporates more tests (94) than "clinical" phenotypic ID systems (20-48)
- More tests = more resolving power - and the ability to accurately identify many more species

## Simplified Test Procedure

- Elimination of variables that can affect results
- **No** Gram-stain necessary to choose test protocol
- **No** pre or post-tests
- **No** additional reagents necessary
- **One minute set-up**

# Phenotype MicroArray Technology

# Tools Characterizing Cellular Traits: Phenotype MicroArrays



**Transcriptomics**

**Proteomics**

**Metabolomics**

**Phenomics**

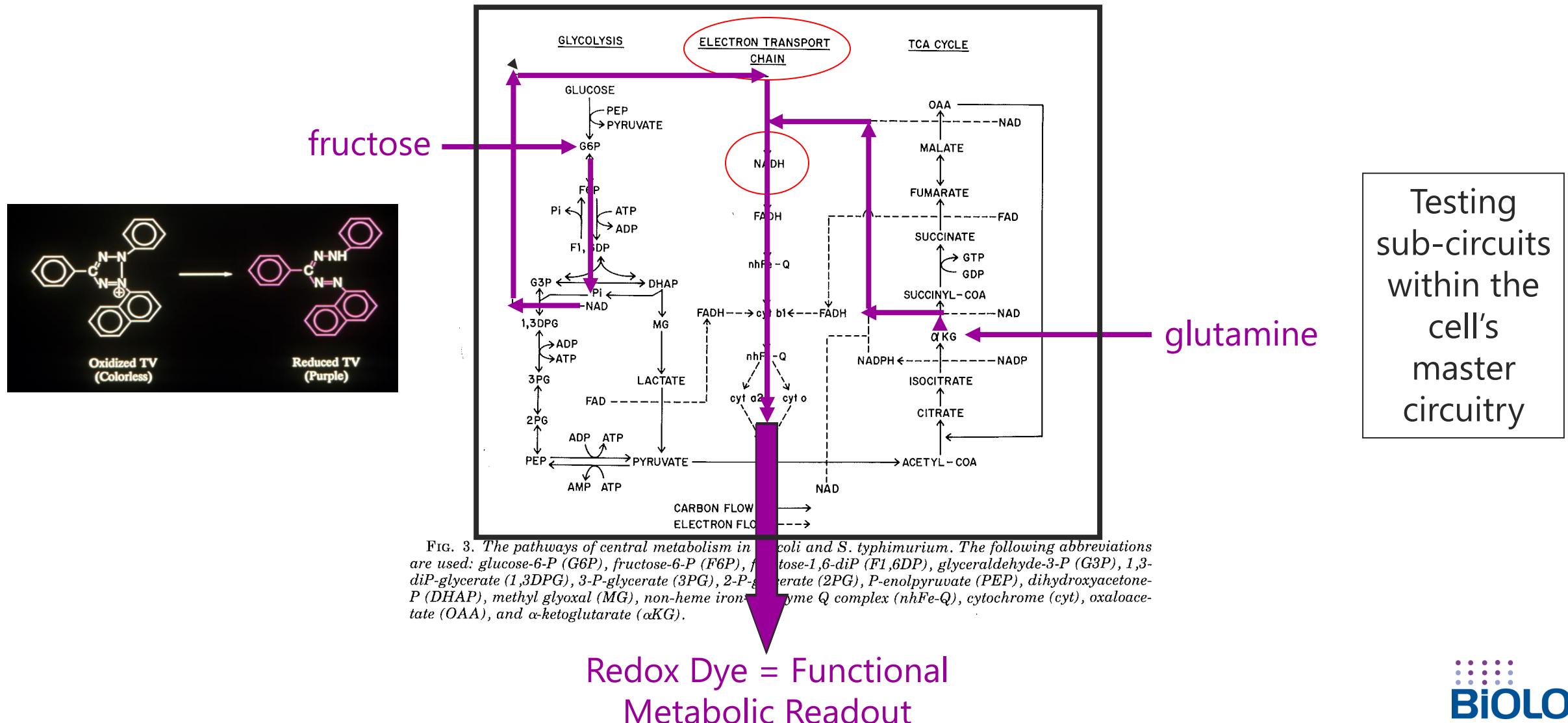
**Molecular Analysis**

**Snap shot** - Characterization of cellular molecules in one growth state, in one instant in time

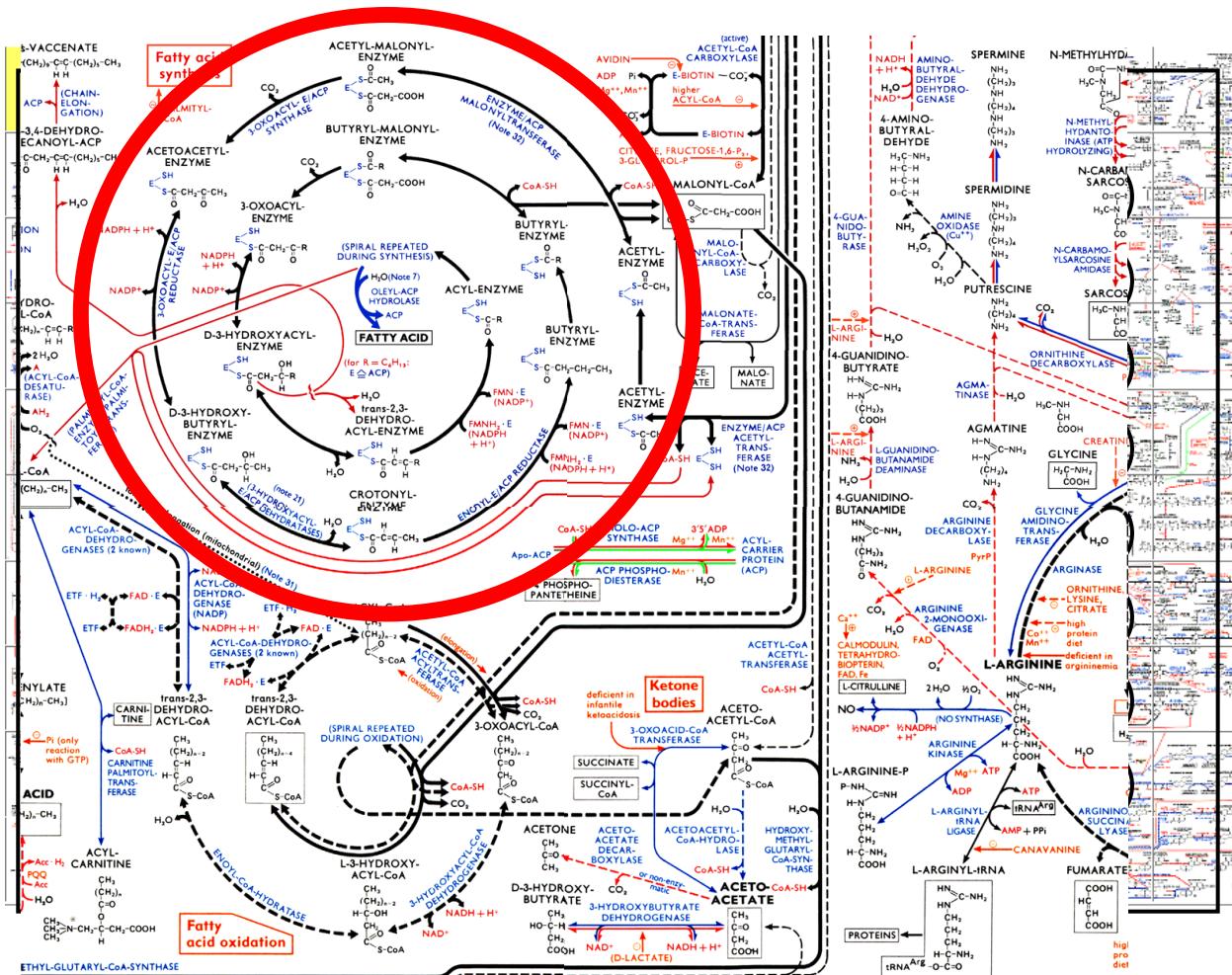
**Cellular Analysis**

**Motion picture** – Record of cellular responses to hundreds of environments over many hours

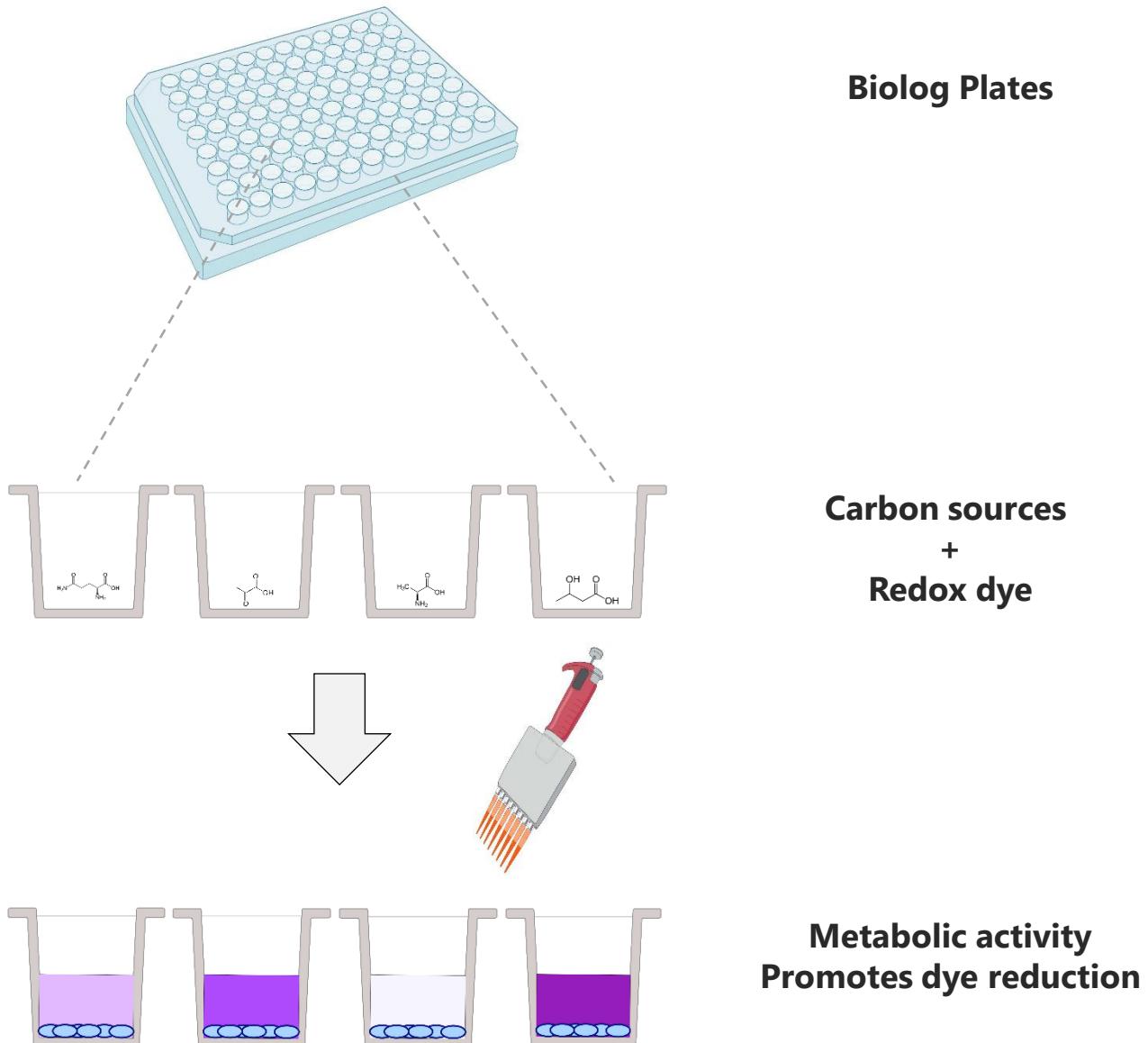
# Metabolism of C-sources produces an electron flow



# Cellular pathways → metabolic phenotypes

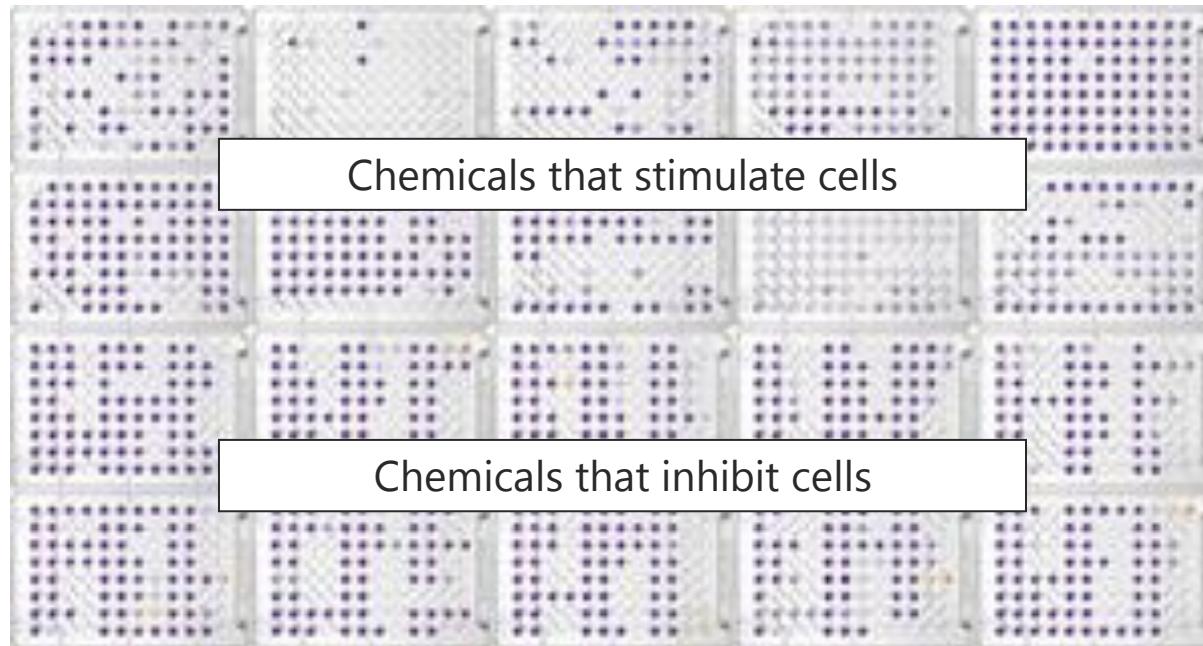


# The active cells contribute to dye reduction



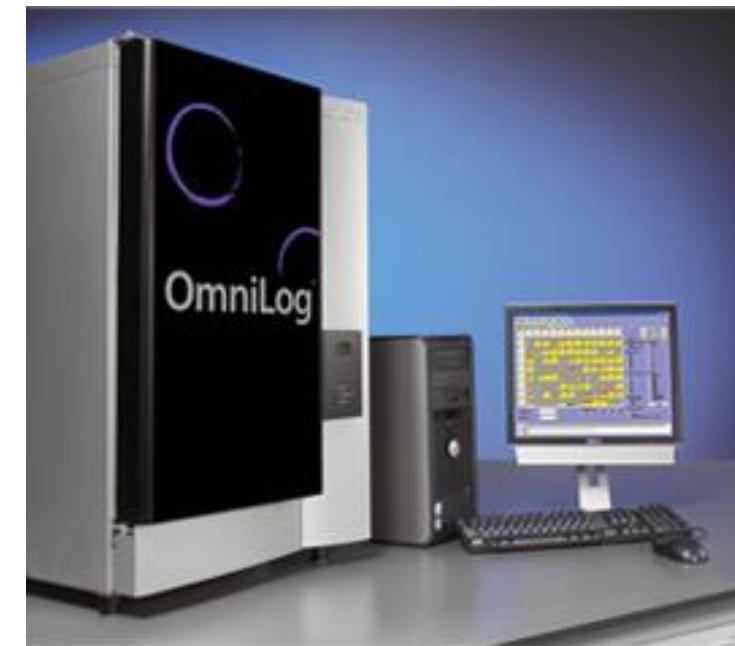
# 2 components of the Biolog cell assay platform

Phenotype MicroArrays



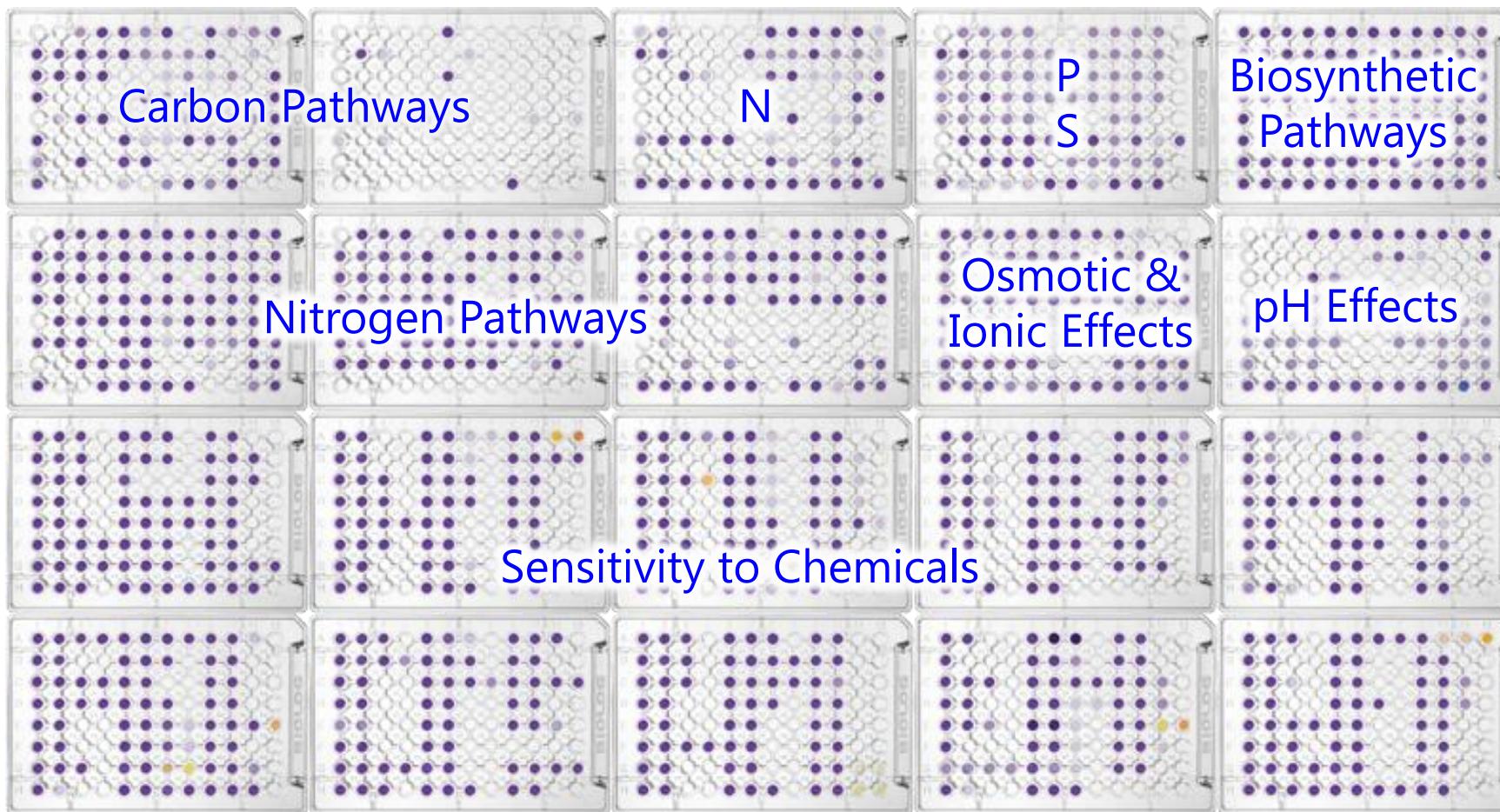
Colorimetric cell assays in 96-well microplates

OmniLog® Incubator/Reader



Incubation and recording of data in the OmniLog

# Phenotype MicroArrays - ~2,000 phenotypic assays

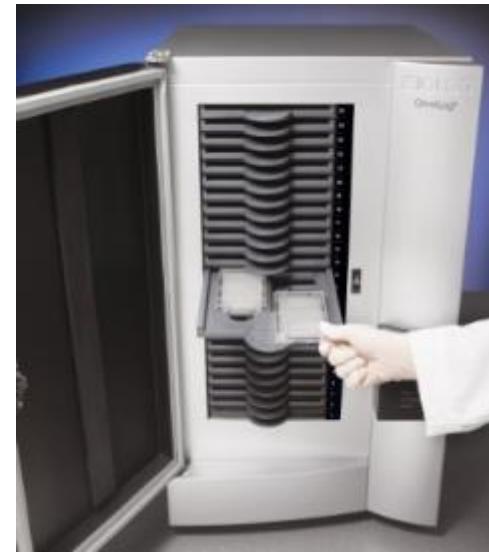


# Phenotype MicroArray assays are easy to run

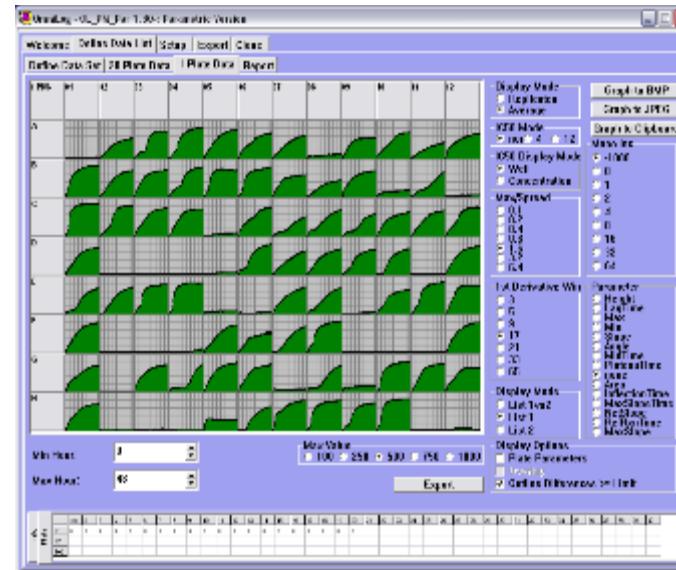
Add cells



Load into OmniLog



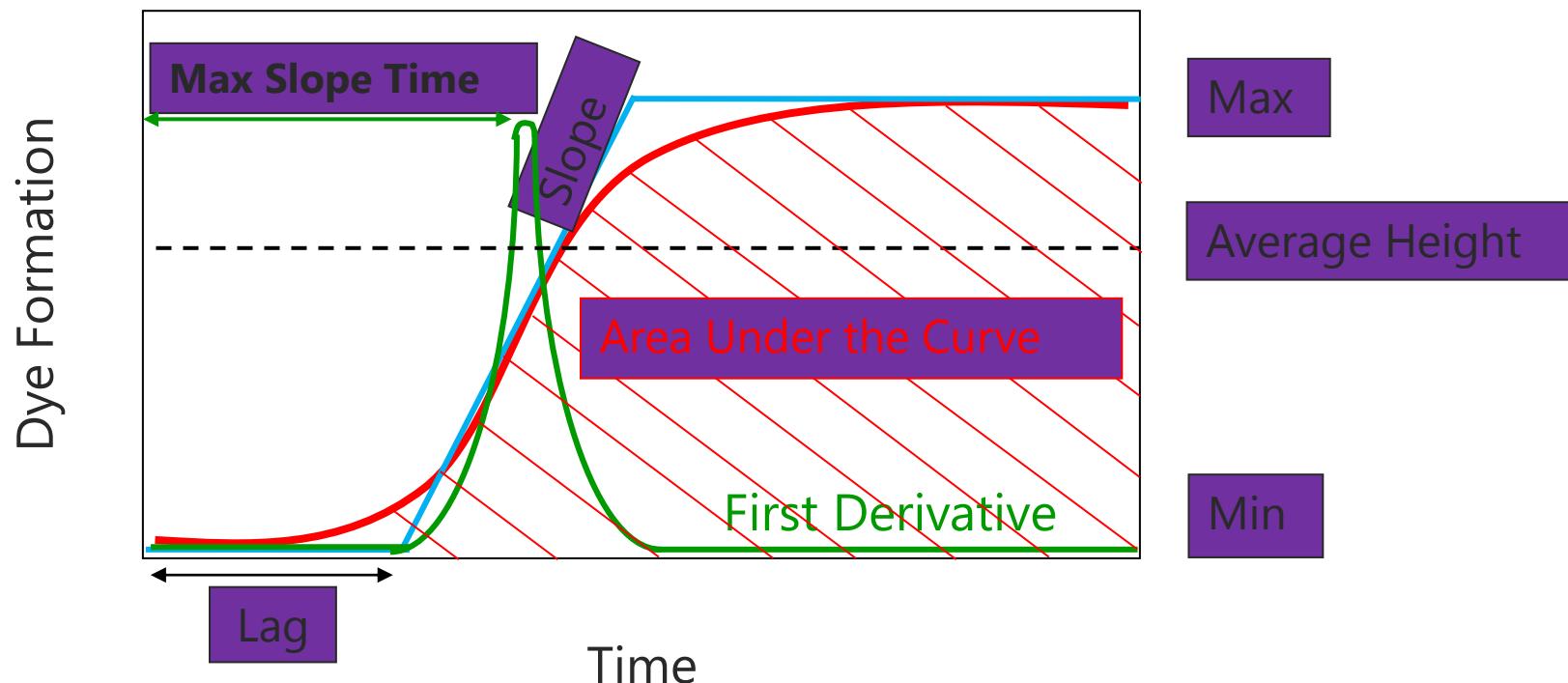
OmniLog records  
**kinetics** from each  
well – either  
respiration or growth



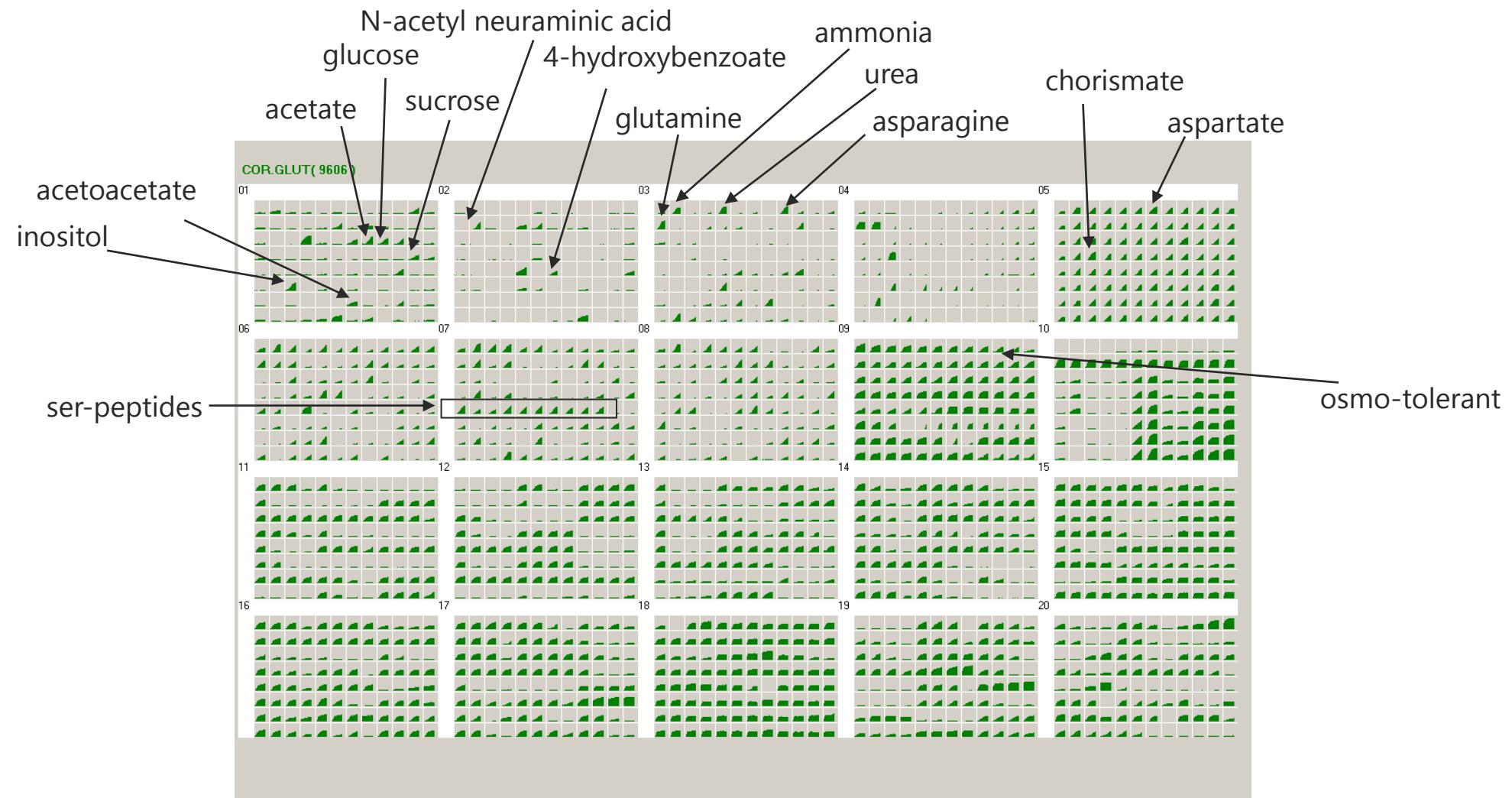
Nearly 5,000 culture  
assays at a time can be  
evaluated

# Additional info from the kinetic curves

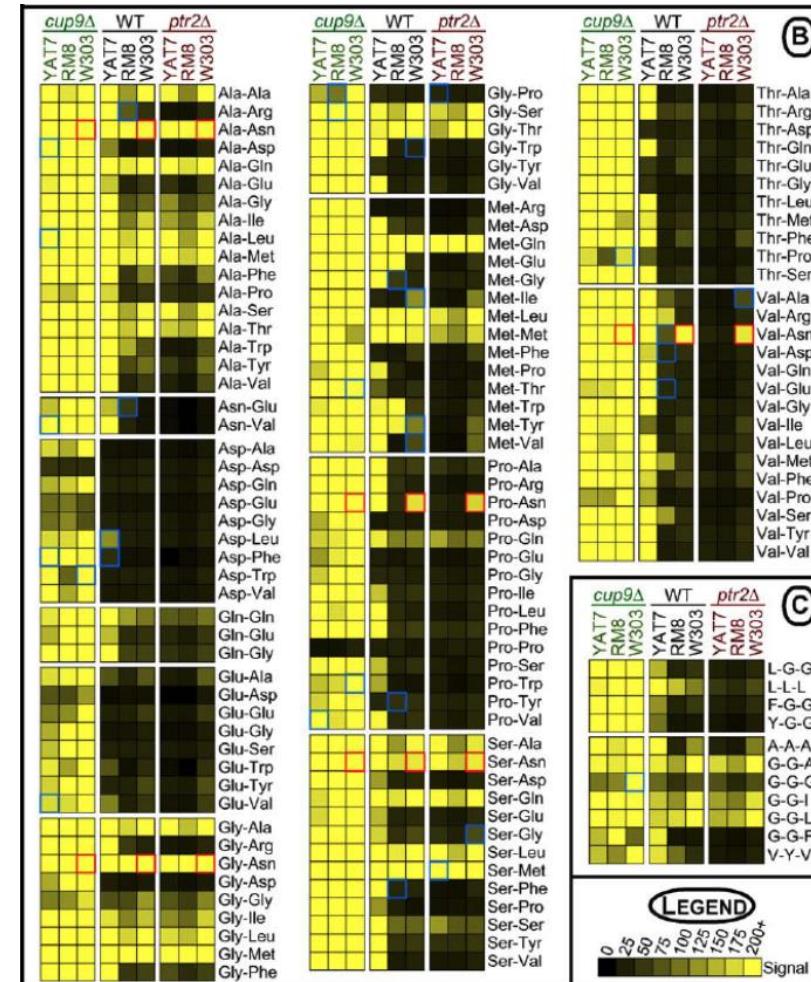
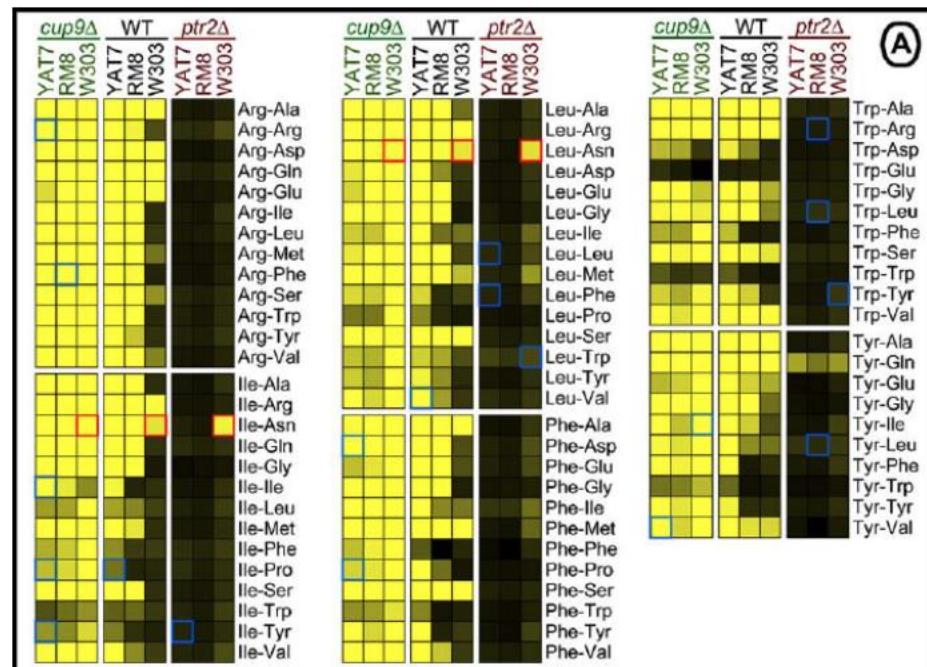
- Each PM well will exhibit a different rate of dye formation, so single endpoint reads for an entire plate are not ideal
- OmniLog PM software computes multiple parameters for phenotypic characterization and comparison



# Phenotype MicroArray analysis of *Corynebacterium glutamicum*



# Analyzing Functional Diversity with Phenotype MicroArrays

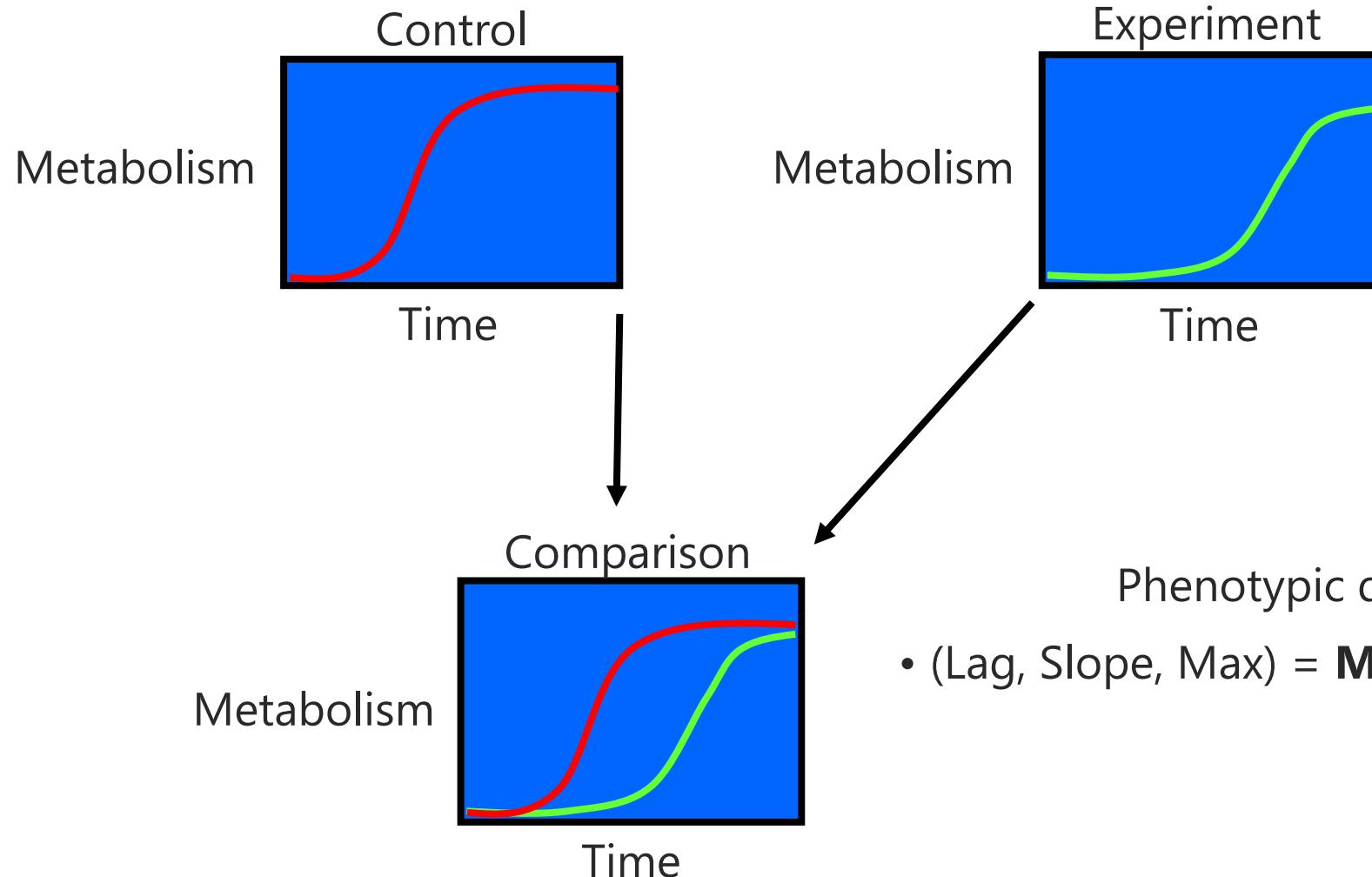


PLoS Genet. 2005 Dec;1(6):e80. Epub 2005 Dec 30.

Harnessing *S. cerevisiae* natural diversity to probe metabolic pathways.

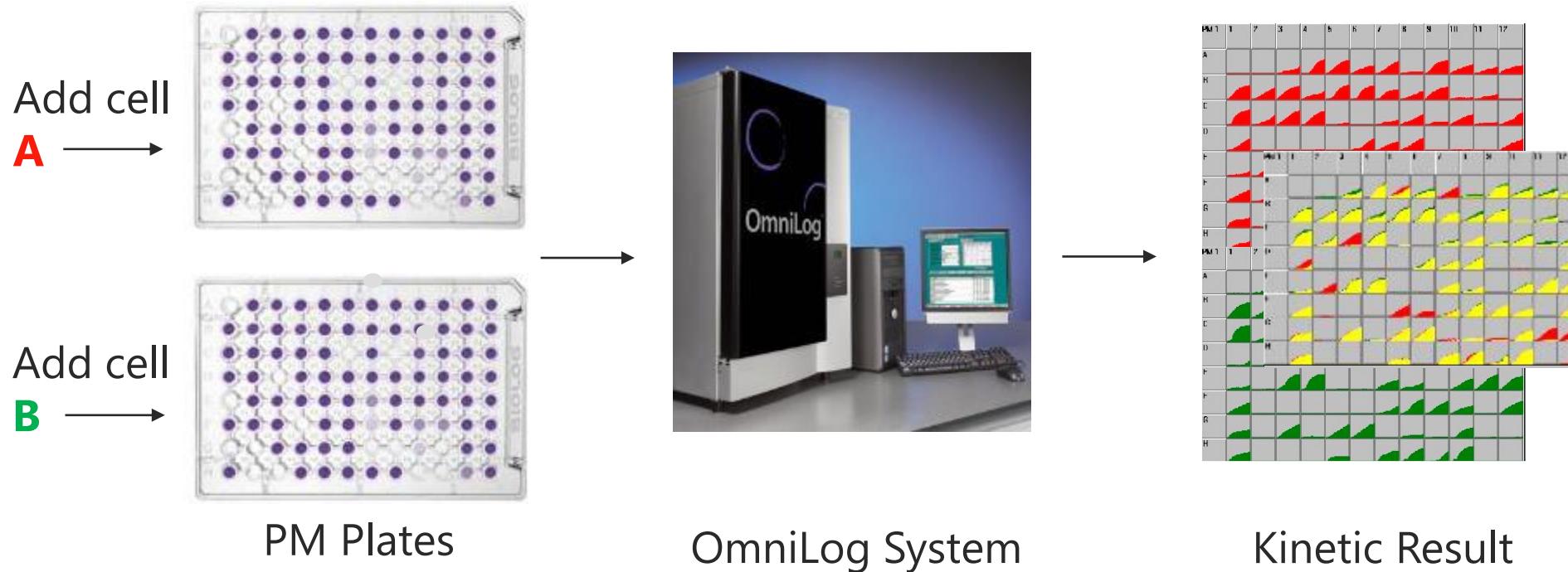
Homann OR, Cai H, Becker JM, Lindquist SL.

# Metabolic curves compared



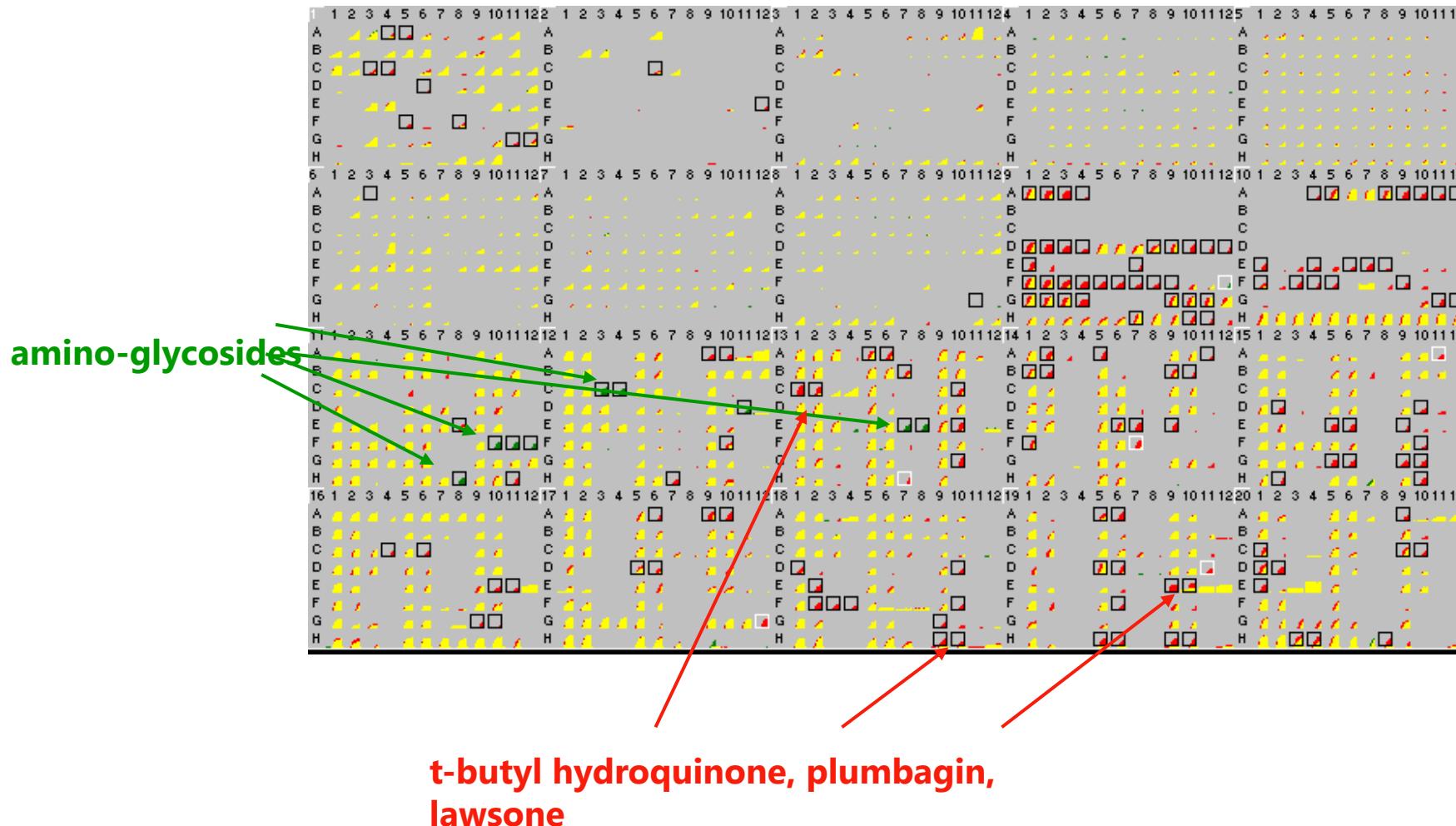
Phenotypic differences:  
• (Lag, Slope, Max) = **Metabolic differences**

# Phenotype MicroArray comparison assays are easy to run



# Knockout comparison

*E. coli* oxyR::kan vs MG1655



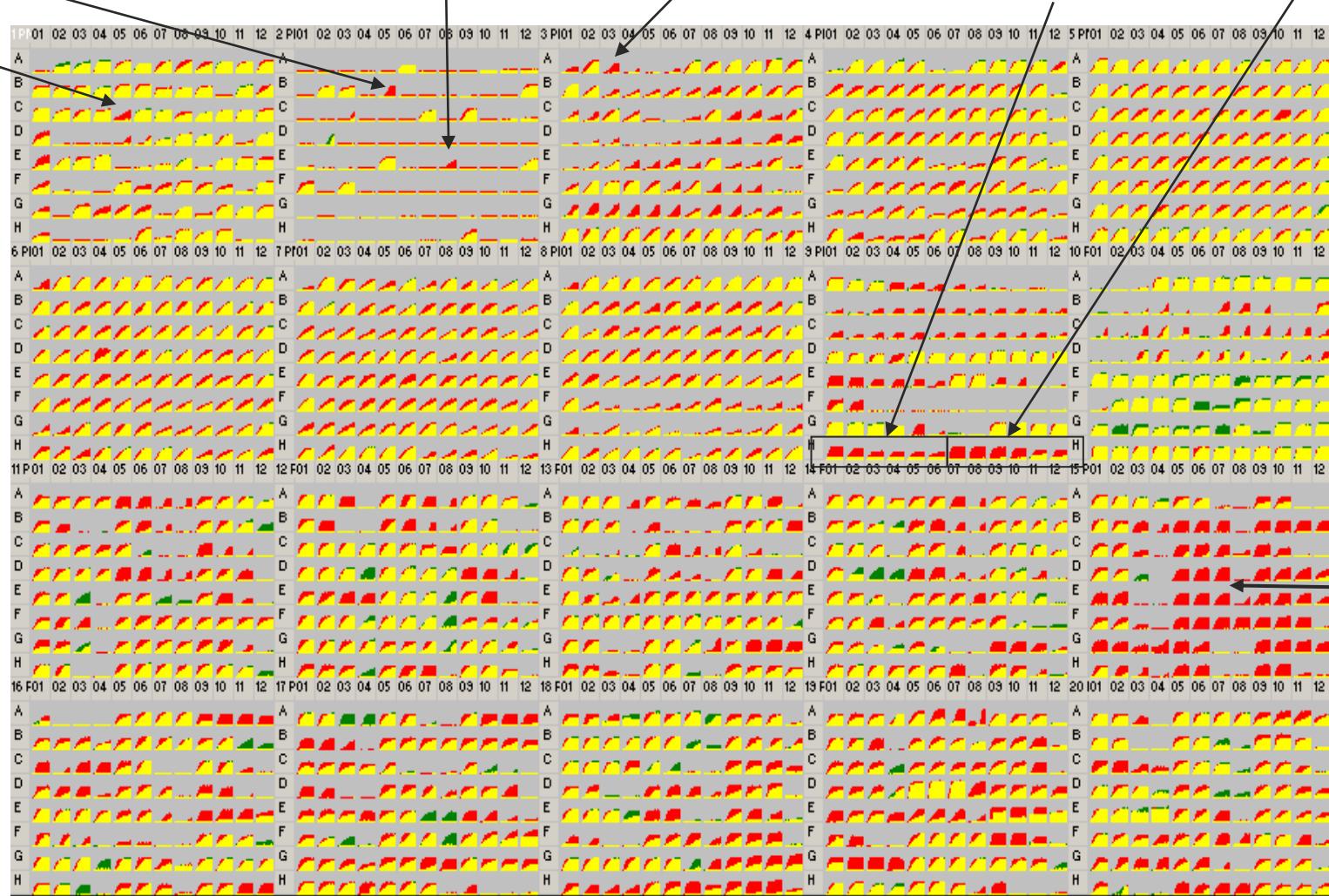
# pH regulation - comparing *E. coli* at pH7 vs pH5

tween 20, D-arabinose,  $\beta$ -hydroxy-butyrate

Nitrite as  
N-source

10-100mM  
Na Nitrate

10-100mM  
Na Nitrite

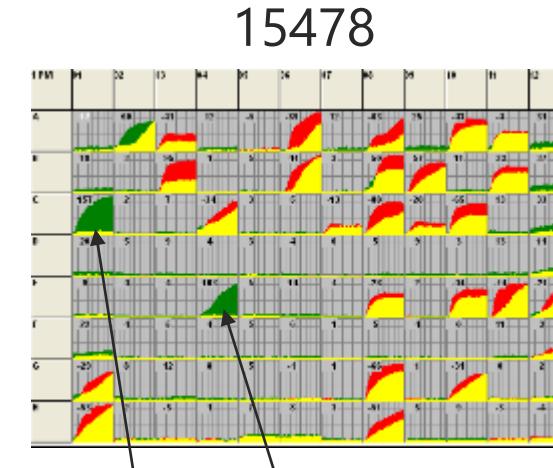
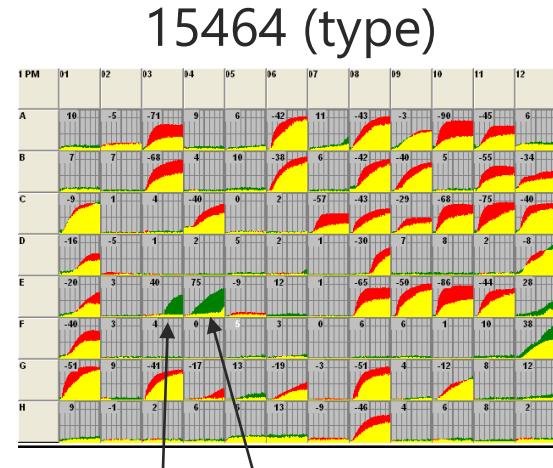
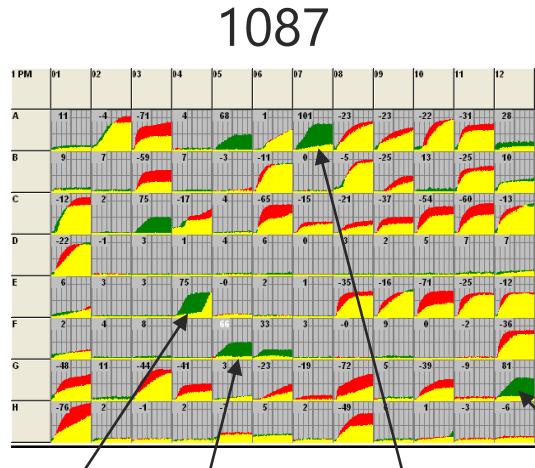


at acidic pH,  $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{HNO}_2$  (nitrous acid) and  $\text{NO}$  (nitric oxide)

# Temperature regulation of carbon metabolism

*Yersinia pseudotuberculosis* at 26° C vs 33° C

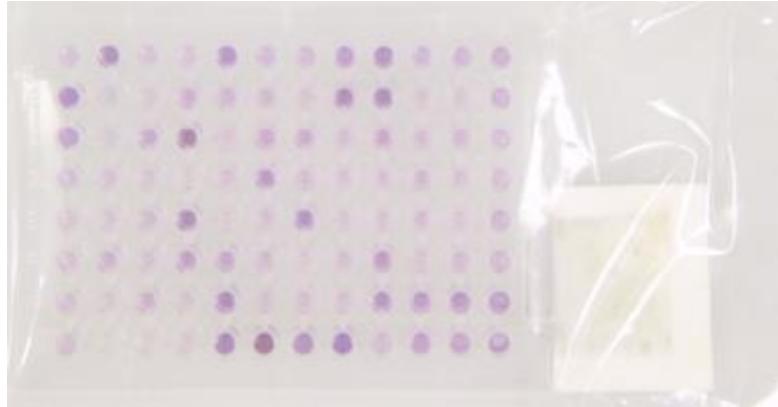
Metabolism of sugar-phosphates is turned on by low temperature



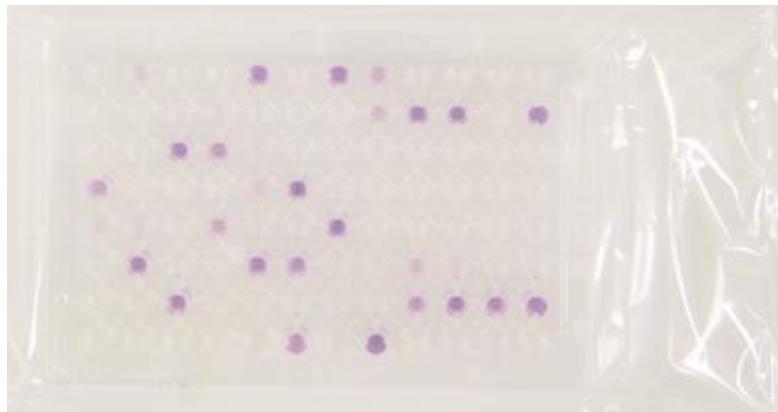
Recent results show that *Yersinia* has a temperature sensing protein, RovA, that is an important regulator of pathogenicity

# Cells can be rested in different gas environments

*Helicobacter pylori*  
HK152

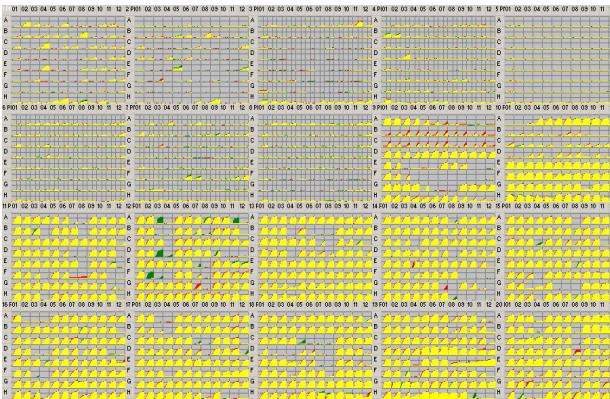


*Campylobacter jejuni*  
NCTC11168



# Easy MOA assessment using drug vs phenotype titration

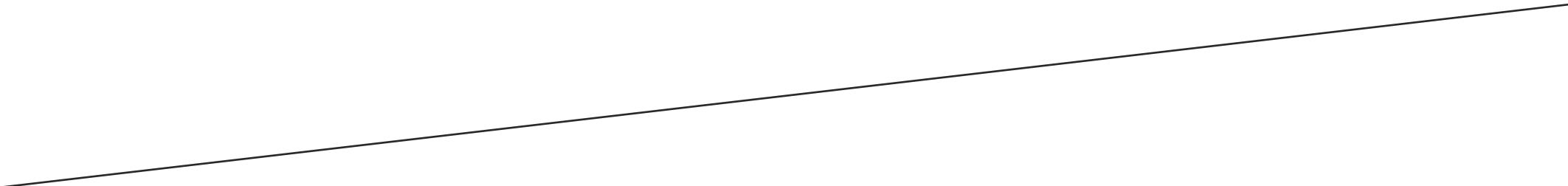
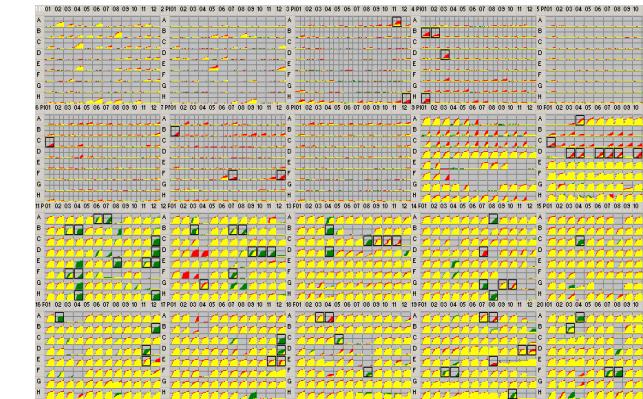
10 $\mu$ M



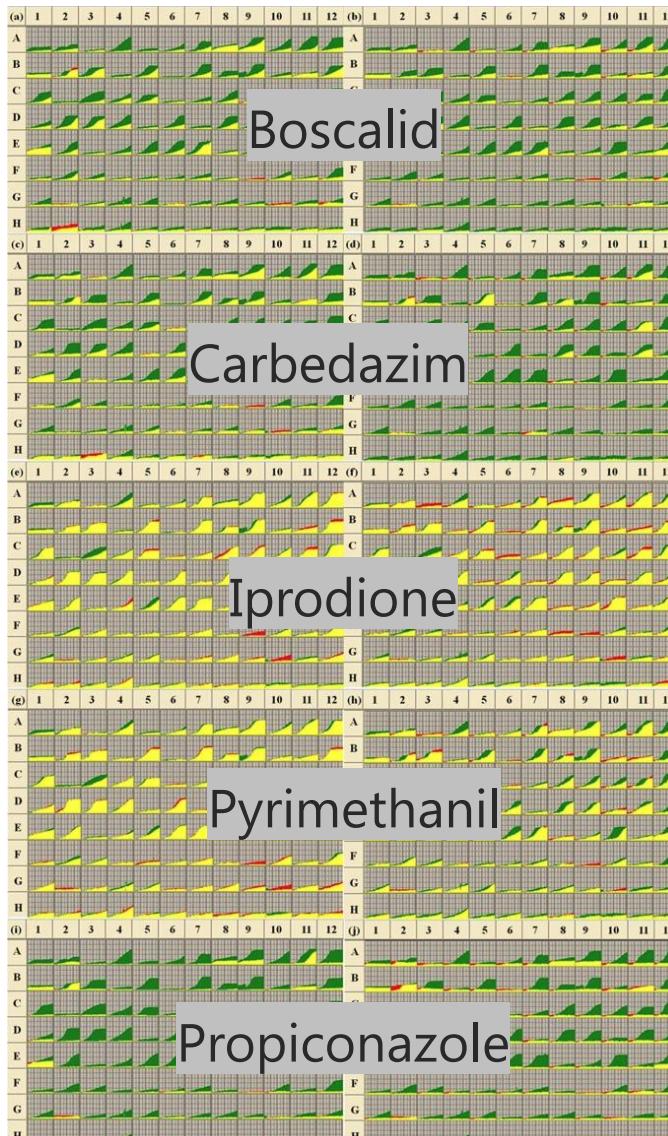
100 $\mu$ M



1000 $\mu$ M



# Metabolic effect of fungicide treatment on *Botrytis cinerea*



Shut down of tricarboxylic acids use

Shut down glycolysis

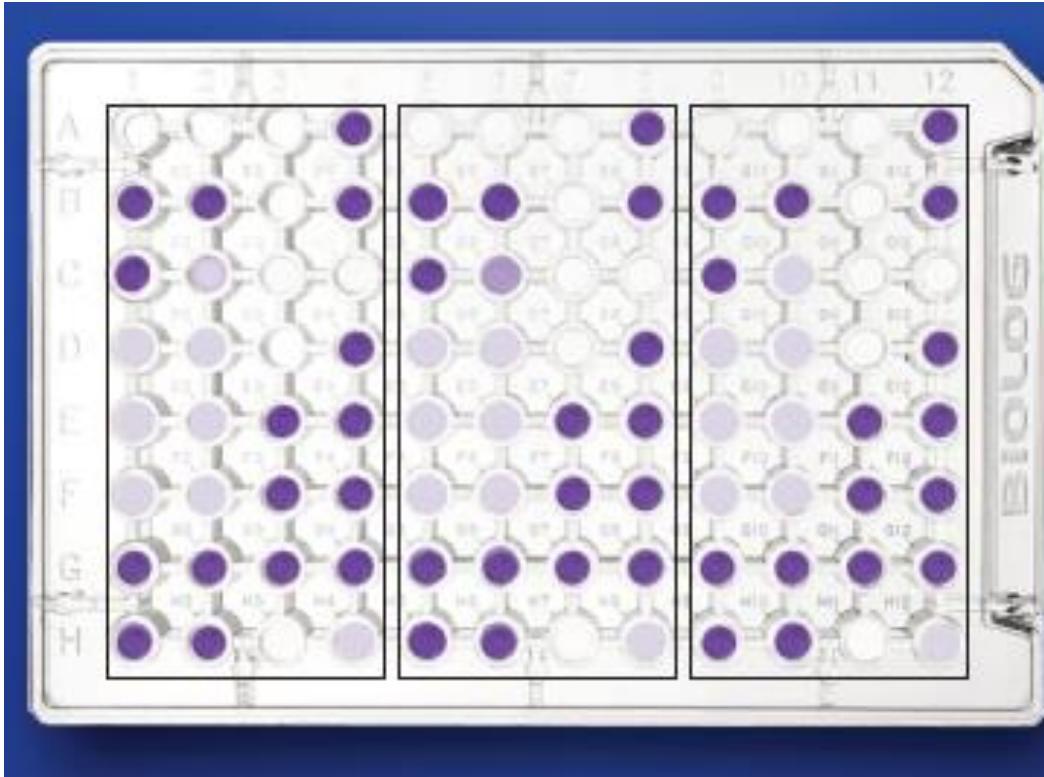
Moderate effect

Almost no effect

Total shutdown

# Functional Analysis of Microbial Communities

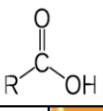
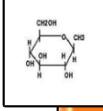
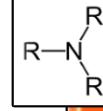
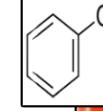
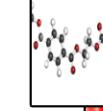
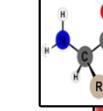
# EcoPlates™



## 31 carbon assays in triplicate

EcoPlates contain 3 repeated sets of 31 carbon sources and employ a tetrazolium redox dye as an indicator of microbial metabolism. As microbes utilize the carbon sources they respiration and the tetrazolium reporter dye is reduced to form a visible purple color. Communities of microorganisms will exhibit a characteristic reaction pattern, a metabolic fingerprint, that reflects the metabolic properties of the community.

# EcoPlate functional guilds/classes

 <b>Carboxylic acids</b>	 <b>Carbohydrates</b>	 <b>Amines</b>	 <b>Phenols</b>	 <b>Polymers</b>	 <b>Aminoacids</b>
<ul style="list-style-type: none"><li>Gammahydroxybutyric acid</li><li>Alphaketobutyric acid</li><li>Galacturonic acid</li><li>Glucosaminic</li><li>Itaconic acid</li><li>Malic acid</li><li>Pyruvic acid</li></ul>	<ul style="list-style-type: none"><li>Cellobiose</li><li>Erthritol</li><li>Galactonic acid lactone</li><li>Acetyl glucosamine</li><li>Glucose 6 phosphate</li><li>Methyl glucoside</li><li>Glycerol phosphate</li><li>Lactose</li><li>Mannitol</li><li>Xylose</li></ul>	<ul style="list-style-type: none"><li>• Phenilethylamine</li><li>• putrescine</li></ul>	<ul style="list-style-type: none"><li>• 2-Hydroxybenzoic acid</li><li>• 4-Hydroxubenzoic acid</li></ul>	<ul style="list-style-type: none"><li>Cyclodextrin</li><li>Glycogen</li><li>Tween 40</li><li>Tween 80</li></ul>	<ul style="list-style-type: none"><li>Arginine</li><li>Asparagine</li><li>Glutamic acid</li><li>Phenilalanine</li><li>Serine</li><li>Threonine</li></ul>

# Using EcoPlates™ with microalgae



Contents lists available at ScienceDirect

Algal Research

journal homepage: [www.elsevier.com/locate/algal](http://www.elsevier.com/locate/algal)

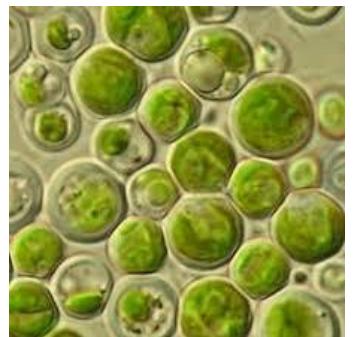


Development of microalgal bioassay based on the community level physiological profiling (CLPP)

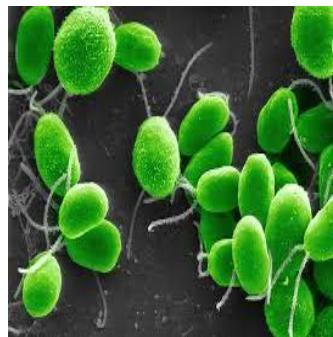


Jun-Woo Kim, Lars Rehmann\*, Madhumita B. Ray\*

Department of Chemical and Biochemical Engineering, University of Western Ontario, 1151 Richmond St, London, Ontario N6A 5B9, Canada



Chlorella  
vulgaris



Chlamidomonas  
reinhardtii



Desmodesmus  
subspicatus

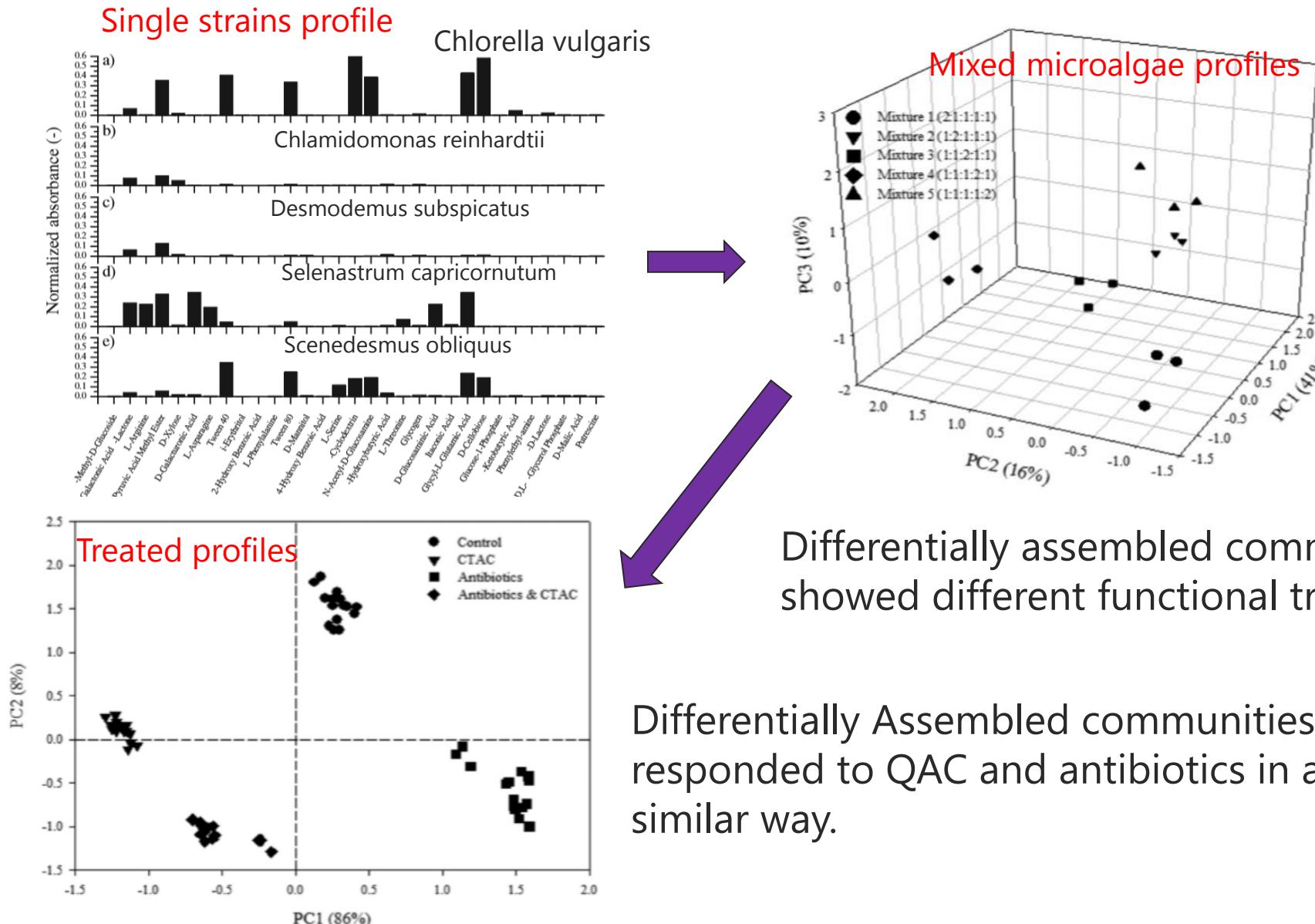


Selenastrum  
capricornutum



Scenedesmus  
obliquus

# Using EcoPlates™ with microalgae



# Employment of EcoPlates™ with anaerobic communities



Article

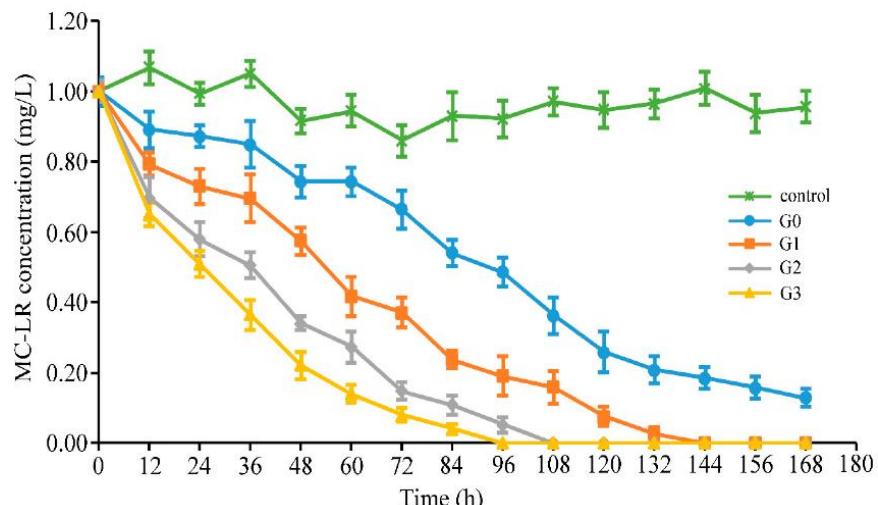
## Effects of Microcystin-LR on Metabolic Functions and Structure Succession of Sediment Bacterial Community under Anaerobic Conditions

Qin Ding <sup>1</sup>✉, Kaiyan Liu <sup>1</sup>, Zhiqian Song <sup>2</sup>, Rongli Sun <sup>1</sup>, Juan Zhang <sup>1</sup>✉, Lihong Yin <sup>1</sup> and Yuepu Pu <sup>1,\*</sup>

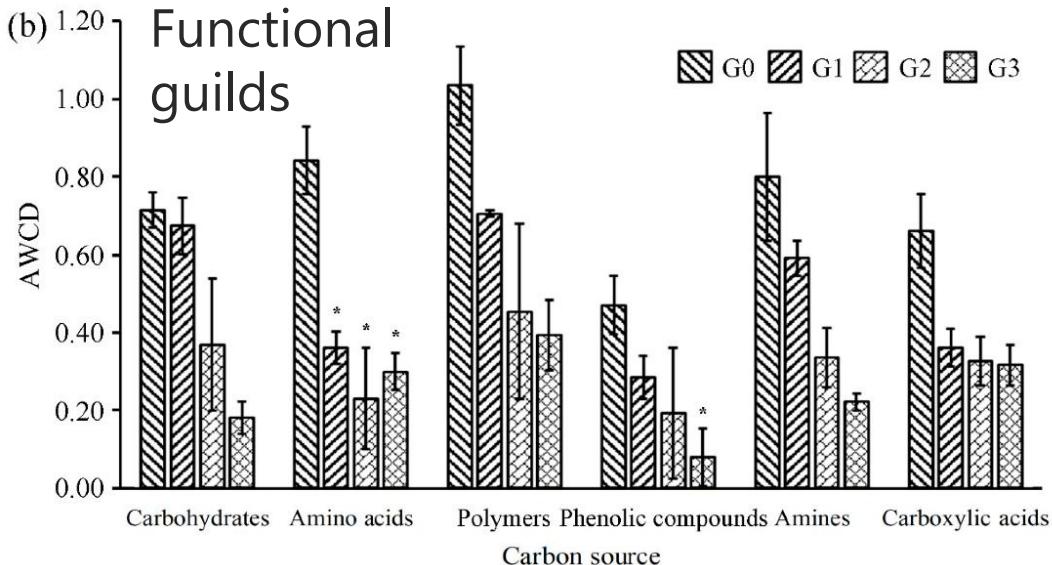
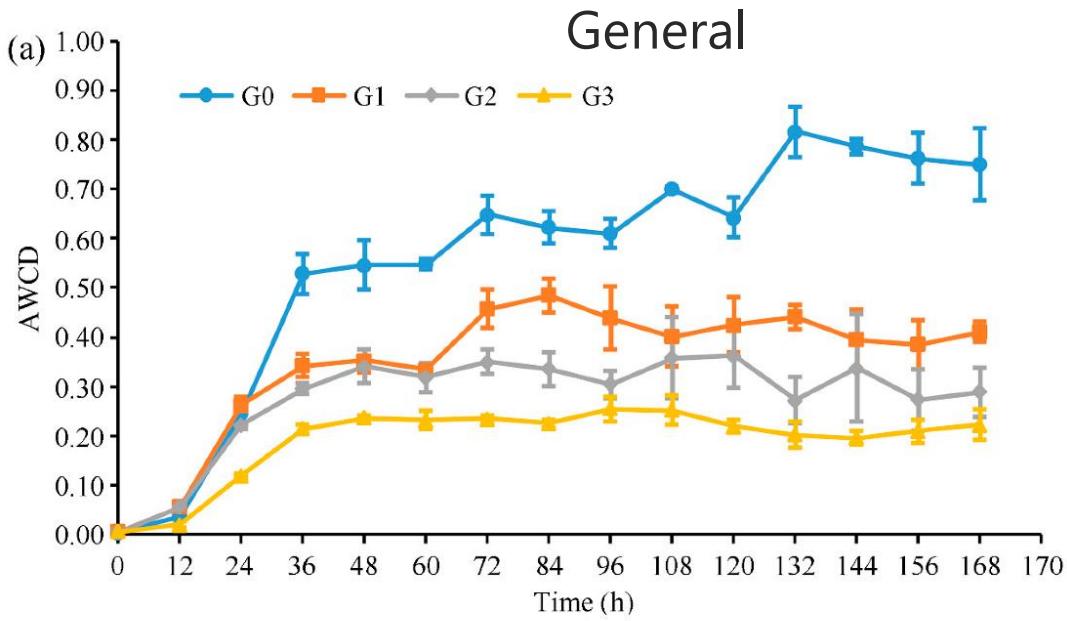


**Cyanotoxin may affect ecosystem service carried out by microbes**

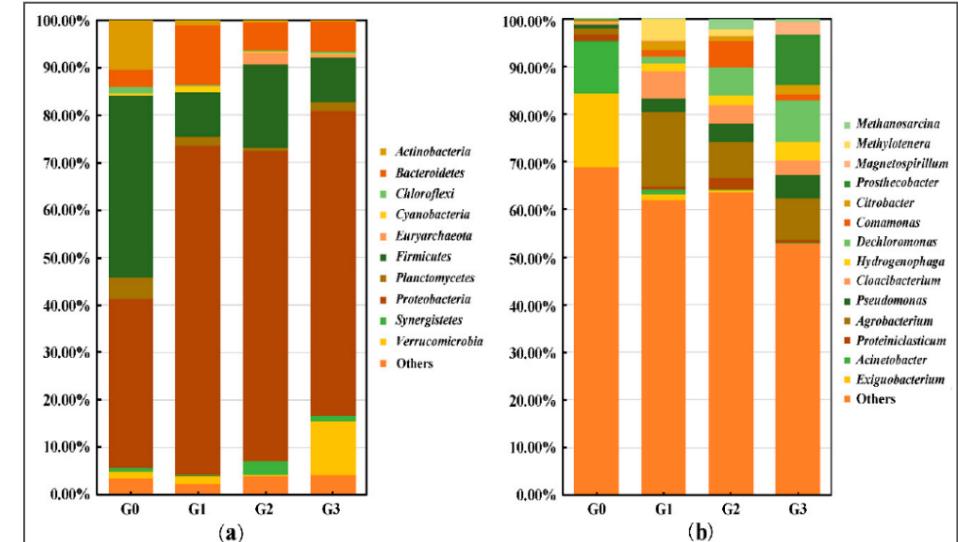
Increased time in contact  
with the cyanotoxin



# Employment of EcoPlate™ with anaerobic communities



Community structure  
(16SrRNA seq)



Overall AWCD was impacted, but some functional guilds more then others

# Biolog solutions for community analysis

## GEN III plates

Used for bacterial ID

pH, antibiotic resistance, osmolarity are tested as well

Can be used with and without dye

## PM plates

Used for metabolic profiling of single strains

Nitrogen metabolism could be assayed

Can be used with and without dye

## FF plates

Used for fungal ID

Carbon sources selected to work with fungi

## AN plates

Used to **ID anaerobes**

**Carbon sources selected for anaerobes**

# Using AN plates with anaerobic communities

OPEN  ACCESS Freely available online

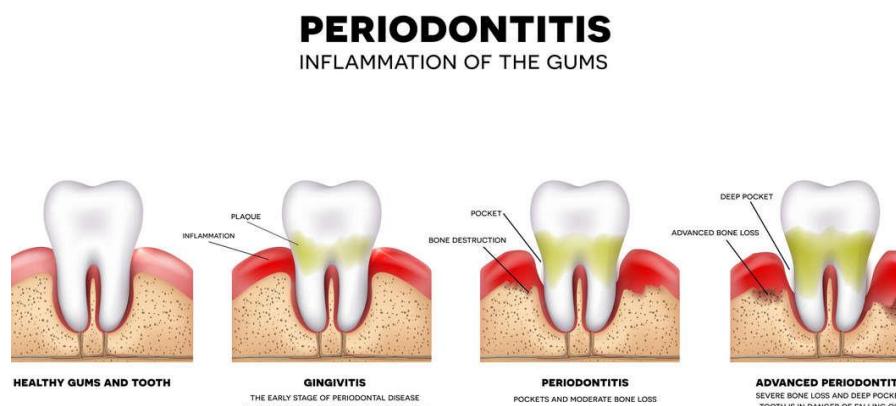
 PLOS ONE

## Functional Diversity of the Microbial Community in Healthy Subjects and Periodontitis Patients Based on Sole Carbon Source Utilization

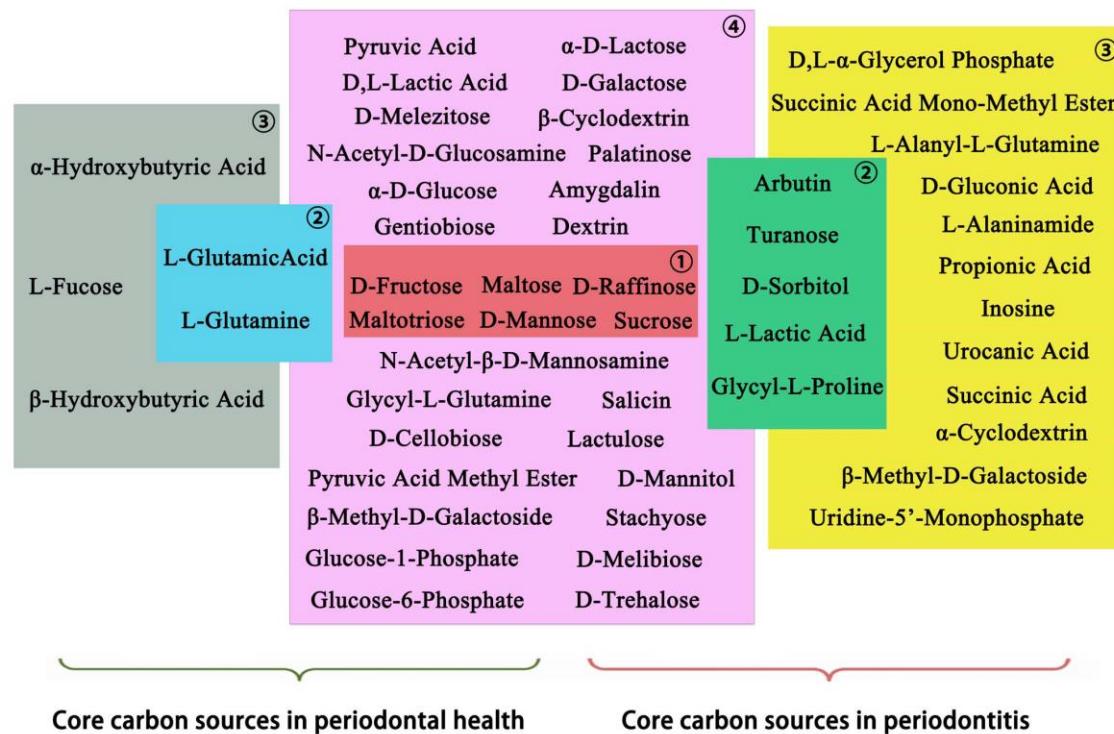
Yifei Zhang<sup>1</sup>\*, Yunfei Zheng<sup>2</sup>\*, Jianwei Hu<sup>1</sup>, Ning Du<sup>1</sup>, Feng Chen<sup>1</sup>\*

**1** Central Laboratory, School of Stomatology, Peking University, Beijing, P. R. China, **2** Department of Periodontology, School of Stomatology, Peking University, Beijing, P. R. China

Can we clearly see a difference between the healthy and the disease?

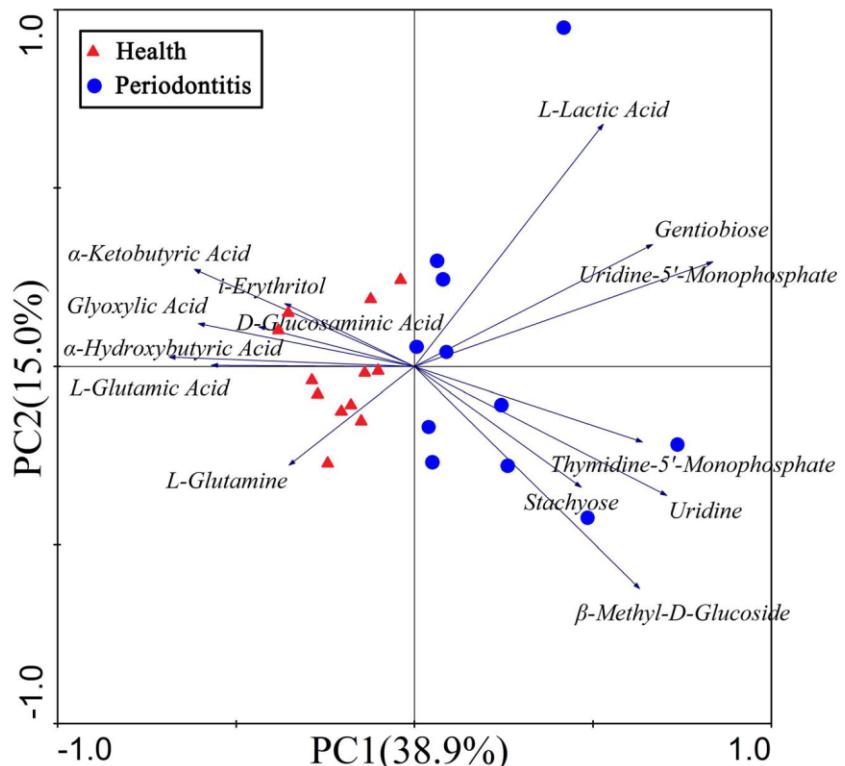


# Normal vs periodontal disease microbial flora



**Figure 2. Core positive carbon sources in the healthy subjects and periodontitis patients.** Inner box (numbered with 1), positive carbon sources found in all subjects (100%); middle boxes (numbered with 2), present in 71–99% of subjects from each group (H: healthy, P: periodontitis); outer boxes (numbered with 3), present in 50–70% of subjects from the healthy and periodontitis groups; middle box (numbered with 4), positive carbon sources present in at least 50% of subjects in both the healthy and periodontitis groups.  
doi:10.1371/journal.pone.0091977.g002

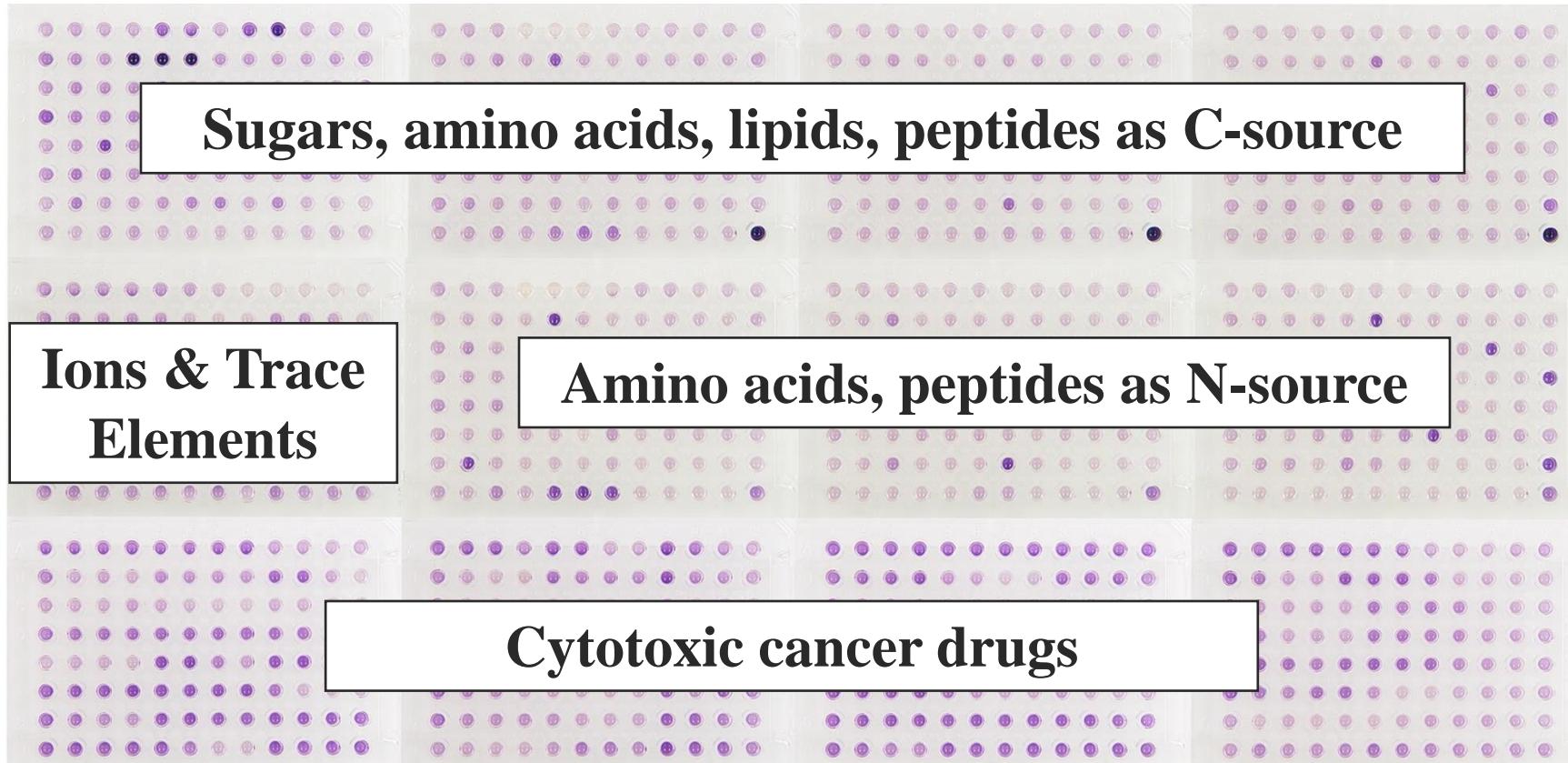
# Normal vs periodontal disease microbial flora



**Figure 5. Ordination biplot of principal component analyses of the substrate utilization patterns of the microbial communities in the healthy and periodontitis groups using Biolog AN plate assays.** Arrows indicate the directions and relative importance (arrow lengths) of the 14 substrates variables.  
doi:10.1371/journal.pone.0091977.g005

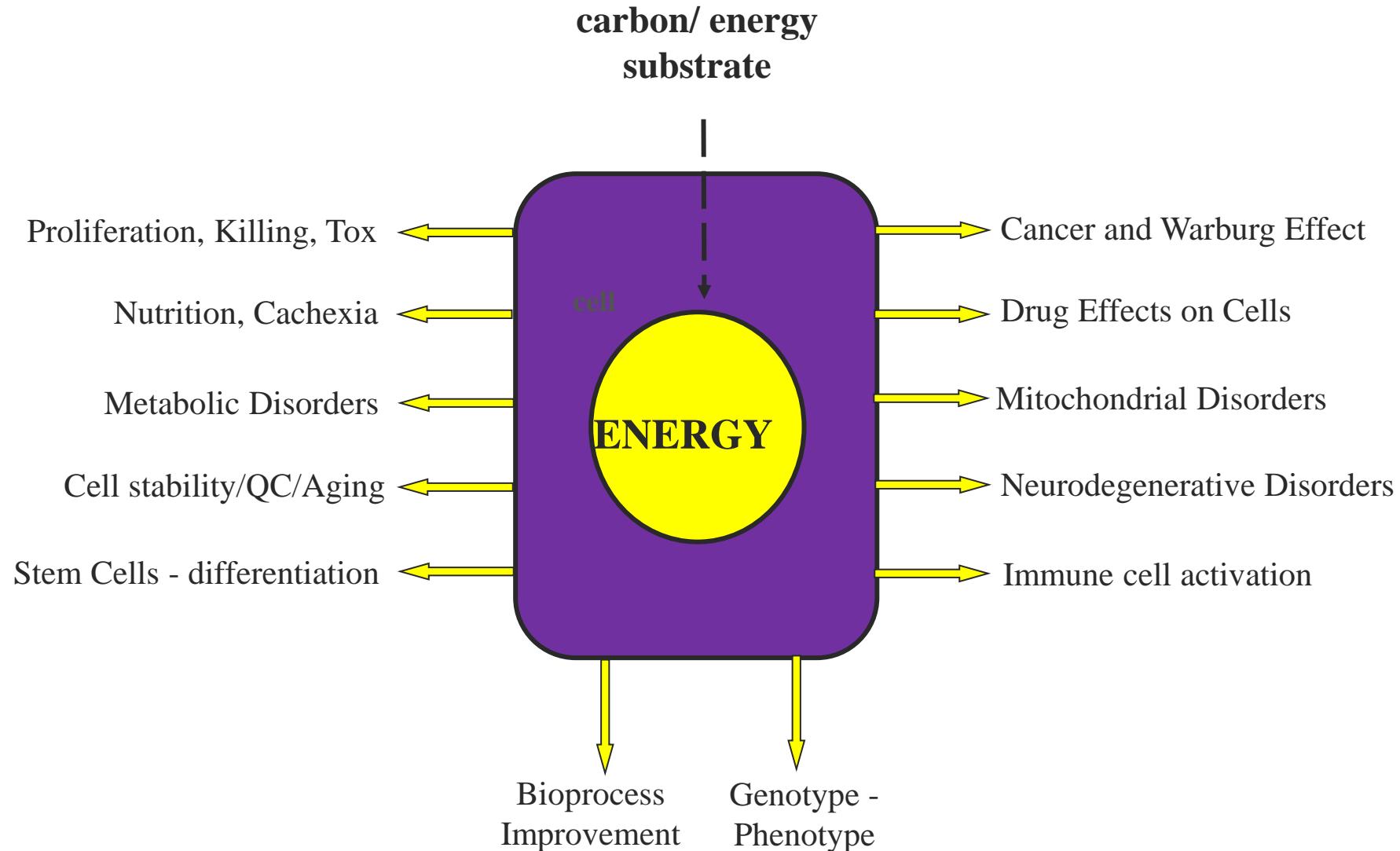
# Beyond Microbes

# **~1500 Assays and Culture Media for Mammalian Cells**



**Plus 3 panels with hormones,  
cytokines and other bioactives**

# Applications of Energy Pathway Assays



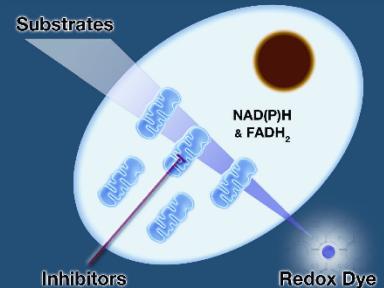
# MitoPlate™ Product Launch

## MitoPlate™ Technology

Analyzing Mitochondria at High Resolution

**New Probes of Mitochondrial Function**

MitoPlates from Biolog provide a powerful new research tool by allowing scientists to run preconfigured sets of 96 mitochondrial function assays in one experiment. Mitochondria can be interrogated and characterized in novel ways, looking at rates of substrate metabolism, sensitivity to drugs and other chemicals, and effects of mutations in mitochondria-related genes.



*Substrates*      *Inhibitors*      *Redox Dye*

*NAD(P)H & FADH<sub>2</sub>*

*Substrates or inhibitors permeate the cell membrane and enter mitochondria, stimulating or inhibiting production of NAD(P)H or FADH<sub>2</sub> which is then measured using a tetrazolium redox dye.*

**Investigate how mitochondria change with:**

- Cell differentiation
- Cancer & ageing
- Neurological disorders
- Metabolic disorders
- Immune cell activation
- Bacterial/viral infection
- Inborn genetic defects

**Assay Principle**

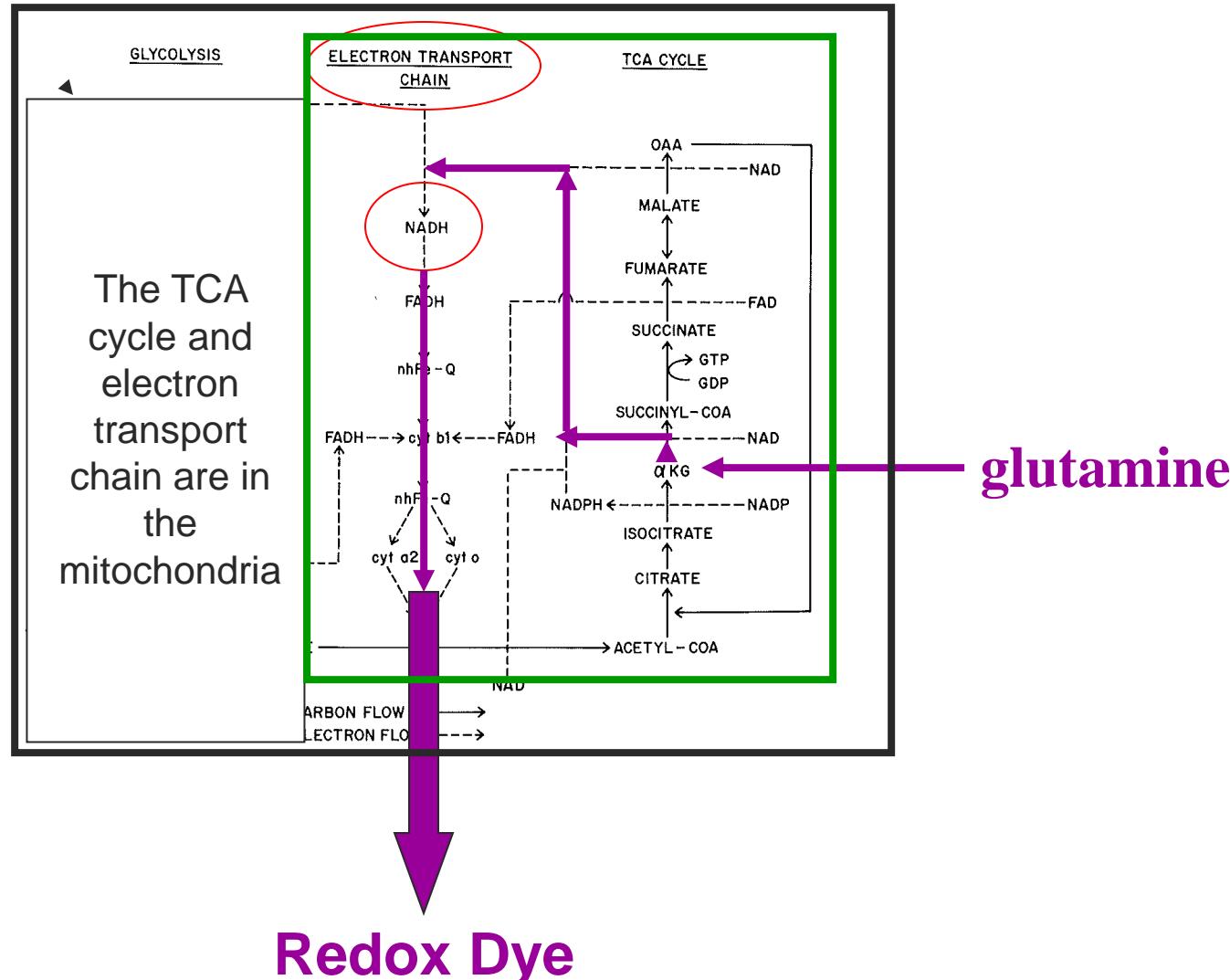
Mitochondrial function is assayed by measuring the rates of electron flow into and through the electron transport chain from metabolic substrates that produce NAD(P)H or FADH<sub>2</sub> such as L-malate, succinate, pyruvate, etc. Each substrate follows a different route, using different transporters to enter the mitochondria and different dehydrogenases to produce NAD(P)H or FADH<sub>2</sub>. The electrons travel from the beginning (complex 1 or 2) to the distal portion of the electron transport chain where a tetrazolium redox dye (MC) acts as a terminal electron acceptor that turns purple upon reduction. Additional MitoPlate assays probe the sensitivity of the mitochondria to a set of 22 diverse inhibitors.

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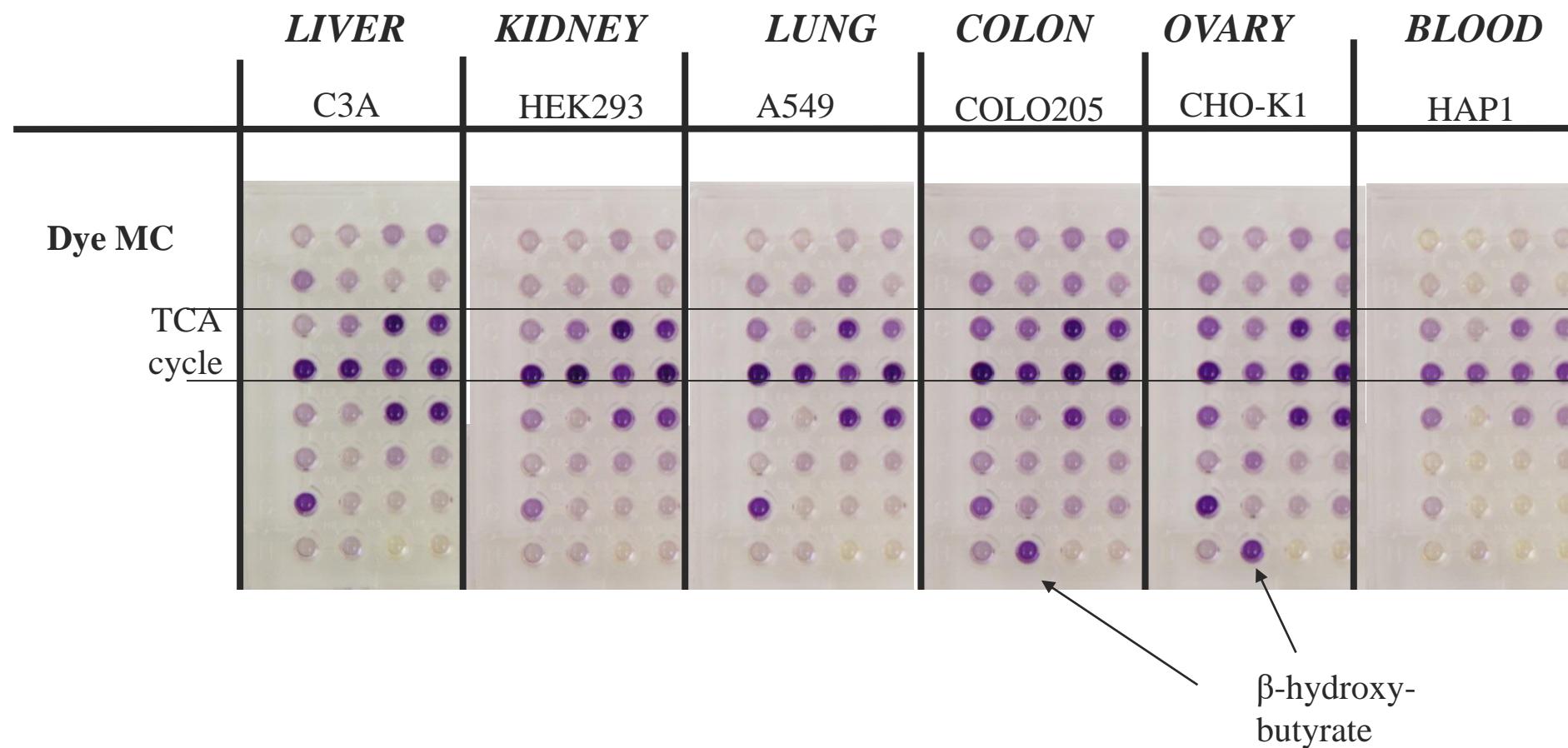
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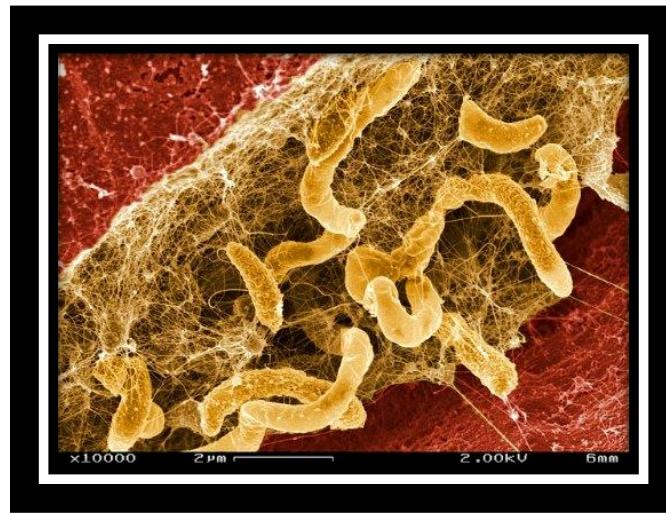
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# Metabolism of Nutrients Produces Cellular Energy



# Comparison of 6 Cell Lines in MitoPlate™ S-1





**Thank you very much for your time!**

Any question? Email [etatti@biolog.com](mailto:etatti@biolog.com)