Metabolism of xenobiotics: Biodegradation and biocatalysis

Dr. Nick Coleman

Microbial metabolism of xenobiotics

Learning outcomes. Be able to…

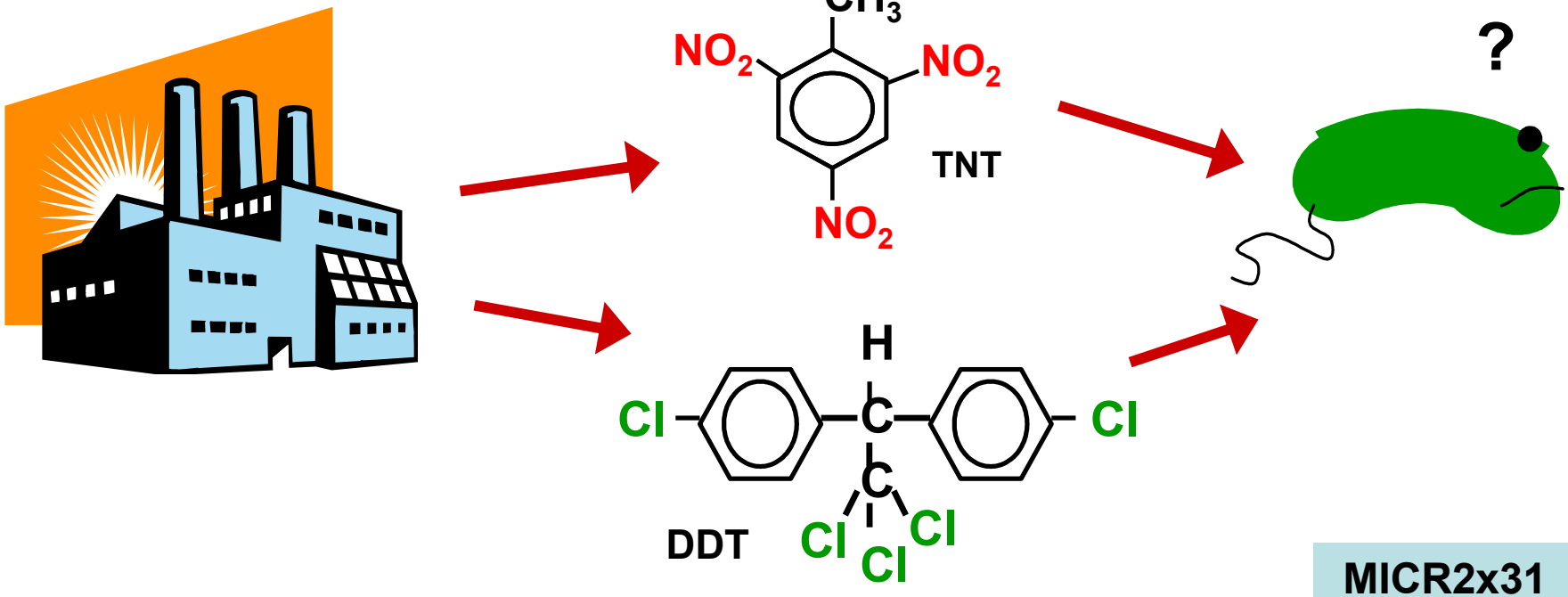
- **Describe the actions of microbes and enzymes in biodegradation of the following pollutants:**
  - **Toluene**
  - **TCE**
  - **PCBs**
- **Explain the different lifestyles and strategies used by aerobic vs. anaerobic pollutant-degraders**
- **Predict the type of biodegradation reactions that would occur for different aromatic and chlorinated pollutants**
- **Explain how enzymes from pollutant-degrading bacteria can be used for biocatalysis**

Biodegradation:

Decomposition of organic compounds by microbes

Recycling via the action of microbial decomposers is an essential step in maintaining functional ecosystems

But pollutant chemicals present a challenge to microbes:



MICR2x31

Xenobiotic:

- a chemical of industrial manufacture, not made by biosynthesis

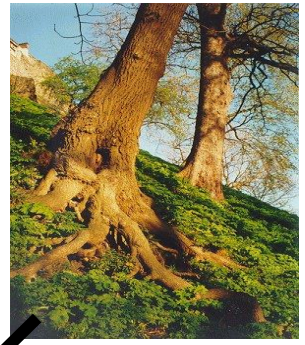
- present in the environment only ~ 100 years
- microbes not yet adapted to them
- recalcitrant to biodegradation.

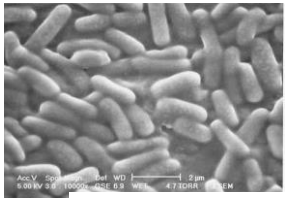
organic chemists

animals

fungi

plants





bacteria

Xenobiotics

Natural compounds

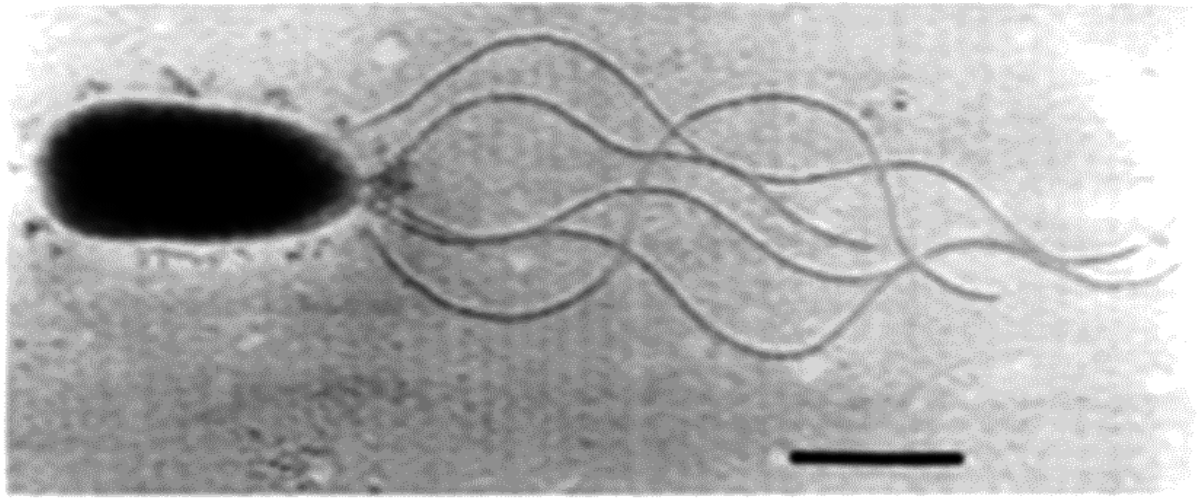
MICR2x31

Part 1. Aerobic biodegradation of aromatics and chlorinated alkenes

Pseudomonas and their dioxygenase

Toluene-degrading bacteria

- ***Pseudomonas putida* F1**
- **Aerobic heterotrophic G-ve rods (Proteobacteria)**
- **Grow on toluene as a carbon and energy source**
- **Requires metabolic genes (*todFC1C2BADEGHI*) and regulator genes (*todST*)**

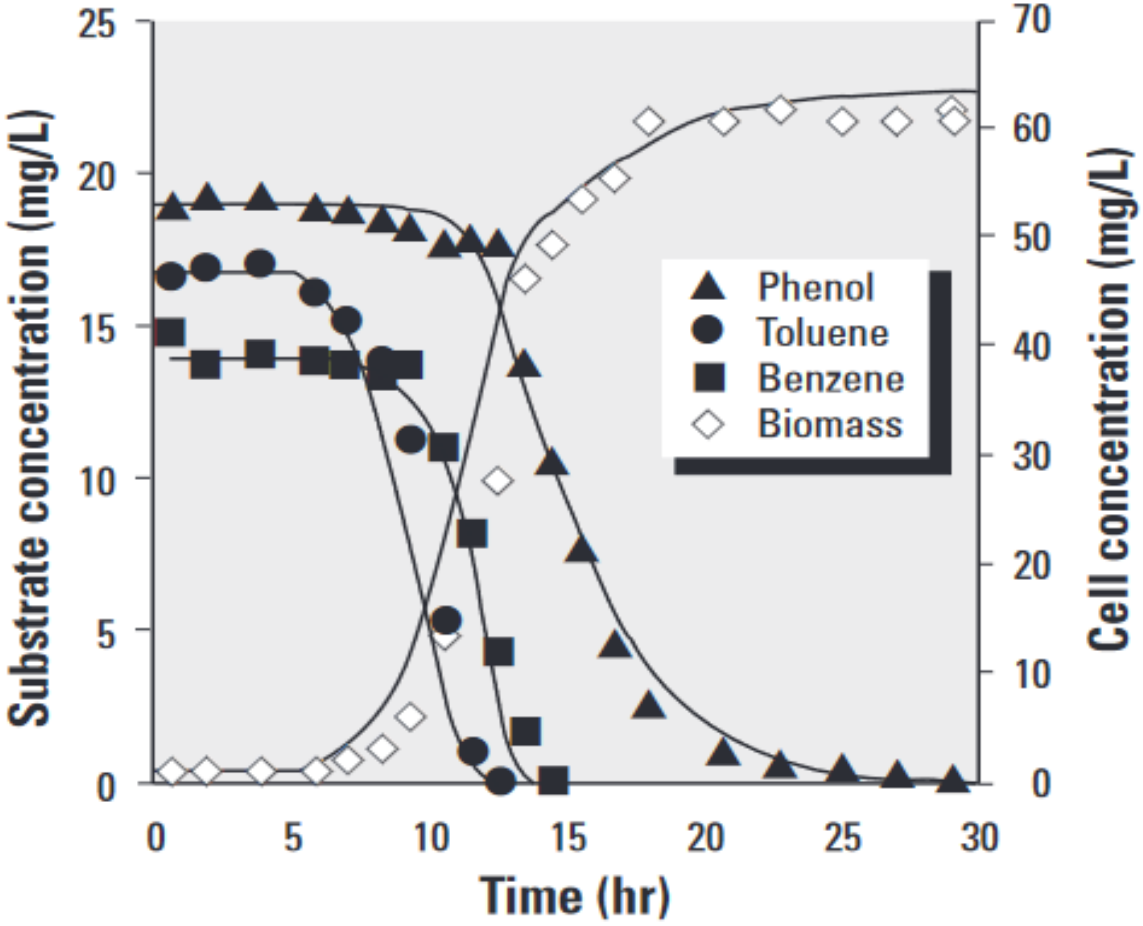


Pseudomonas putida Bar = 1 µm
Harwood et al. 1989. J. Bacteriol. 171:4063-4066.

we will use PpF1 as a positive control in MICR3052 prac

Toluene-degrading bacteria – PpF1

- **PpF1 can grow on toluene, benzene and phenol as carbon sources, separately and together**

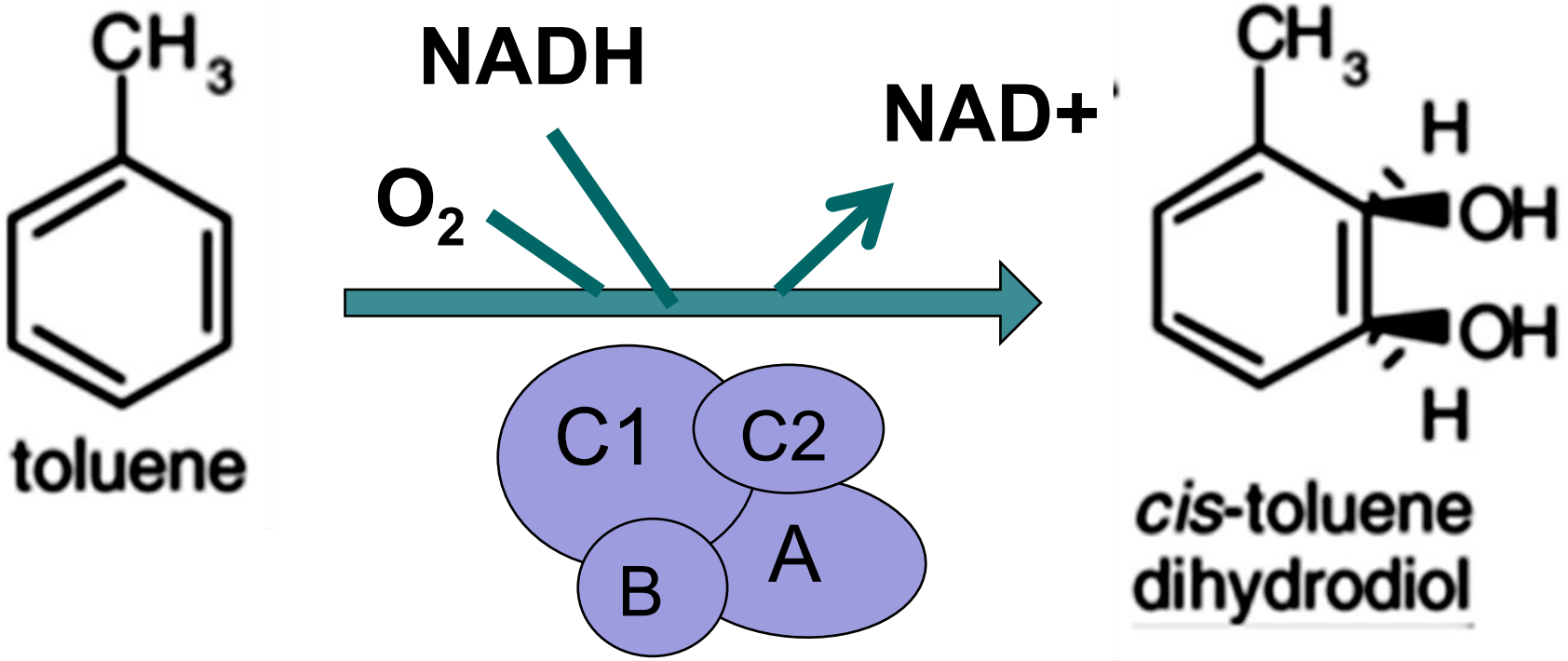


$$\mu = \frac{\mu_{max,1} S_1}{K_{S,1} + S_1 + I_{2,1} S_2} + \frac{\mu_{max,2} S_2}{K_{S,2} + S_2 + I_{1,2} S_1}$$

Environmental Health Perspectives • VOLUME 110 | SUPPLEMENT 6 | DECEMBER 2002

Toluene dioxygenase of PpF1

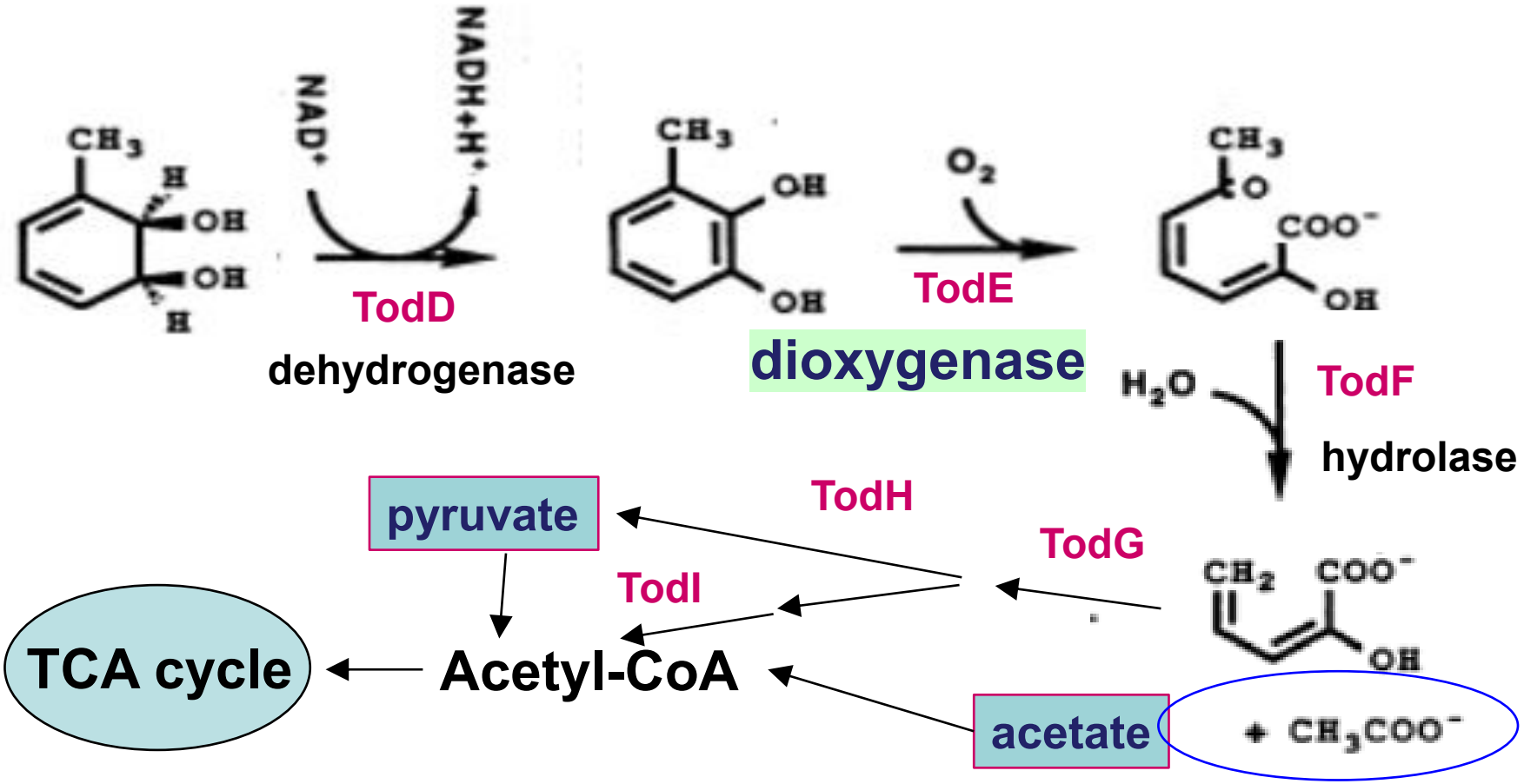
- **The first enzyme in the toluene degradation pathway is a 4-component dioxygenase (TodC1C2BA) that adds oxygen atoms into the toluene aromatic ring**



TodC1C2BA enzyme

Toluene-degradation pathway

- Later steps involve six more enzymes, including another dioxygenase, to yield acetate and pyruvate
- Toluene degradation pathway feeds into TCA cycle

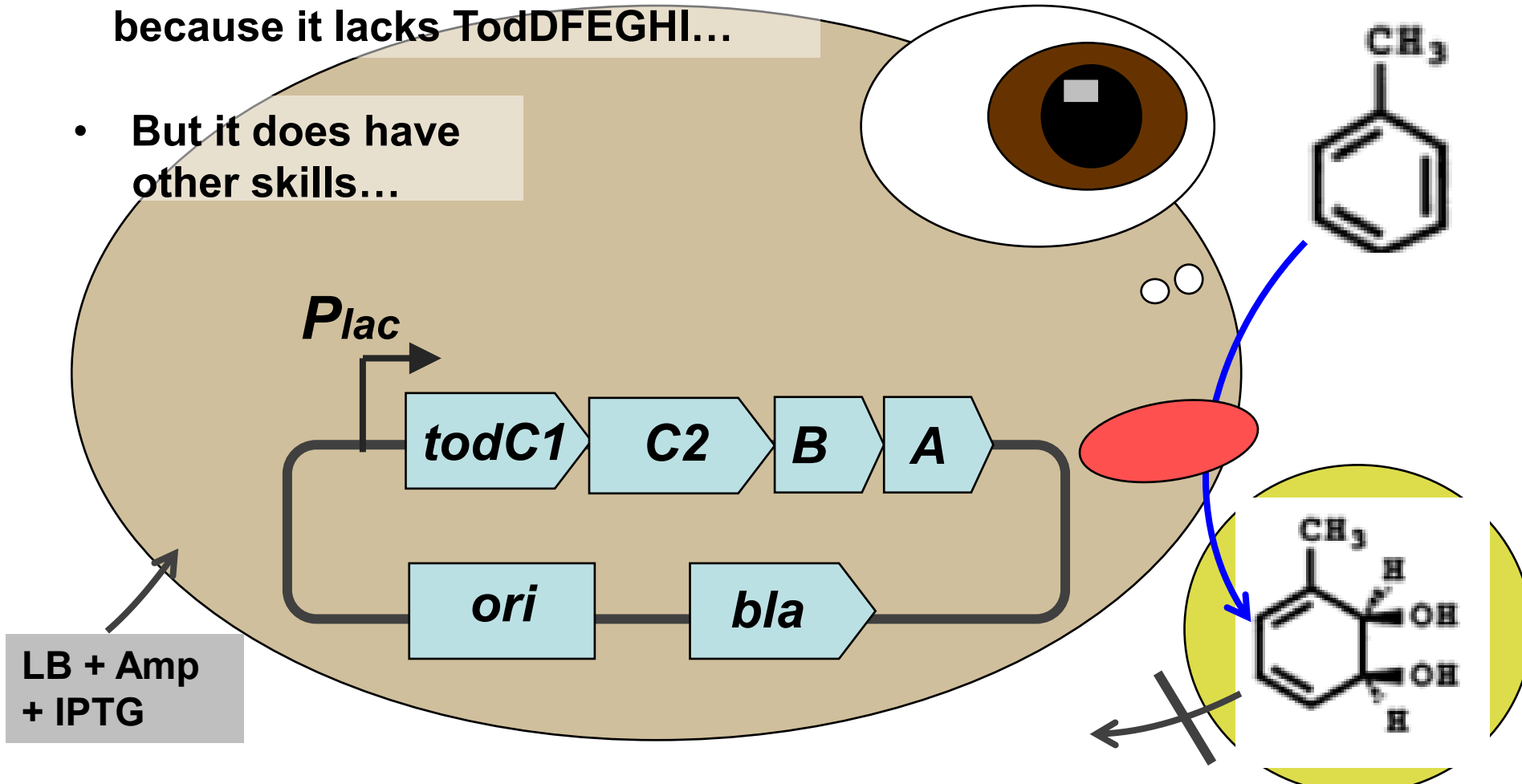


Toluene dioxygenase ... heterologous expression

- **Toluene dioxygenase enzyme TodC1C2BA can be expressed on plasmids in E.coli and other bacteria**

  - **This E.coli can't grow on toluene, because it lacks TodDFEGHI…**

  - **But it does have other skills…**



Toluene dioxygenase

THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1989 by The American Society for Biochemistry and Molecular Biology, Inc.
Vol. 264, No. 25, Issue of September 5, pp. 14940–14946, 1989
Printed in U.S.A.

Toluene Degradation by *Pseudomonas putida* F1

NUCLEOTIDE SEQUENCE OF THE *todC1C2BADE* GENES AND THEIR EXPRESSION IN *ESCHERICHIA COLI**

J.Biol.Chem. (1989) 264:14940-14946

Gerben J. Zylstra and David T. Gibson‡
From the Department of Microbiology and Biocatalysis Research Group, the University of Iowa, Iowa City, Iowa 52242

- **SDS-PAGE to detect expression of TodC1C2BADE proteins in E.coli**

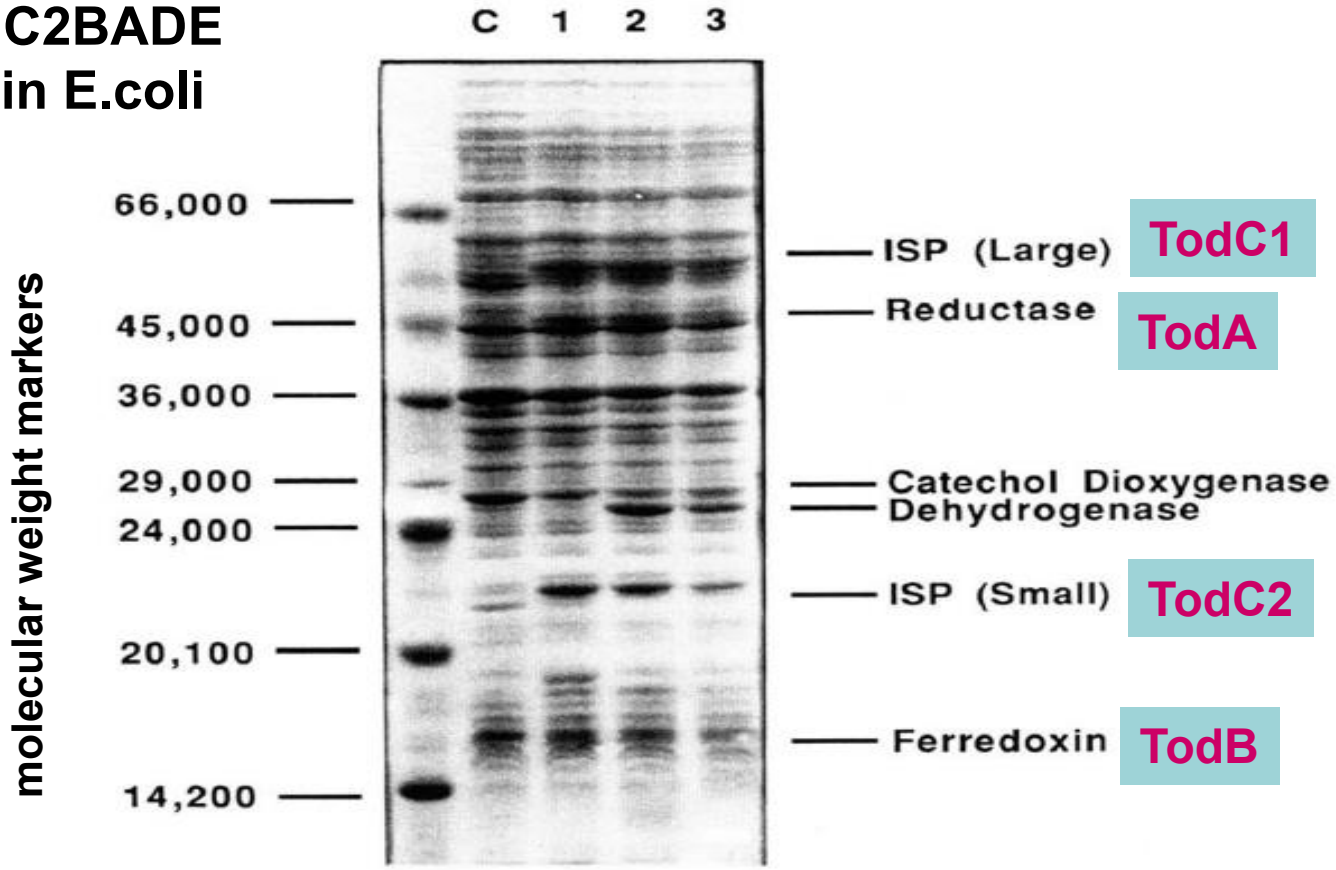
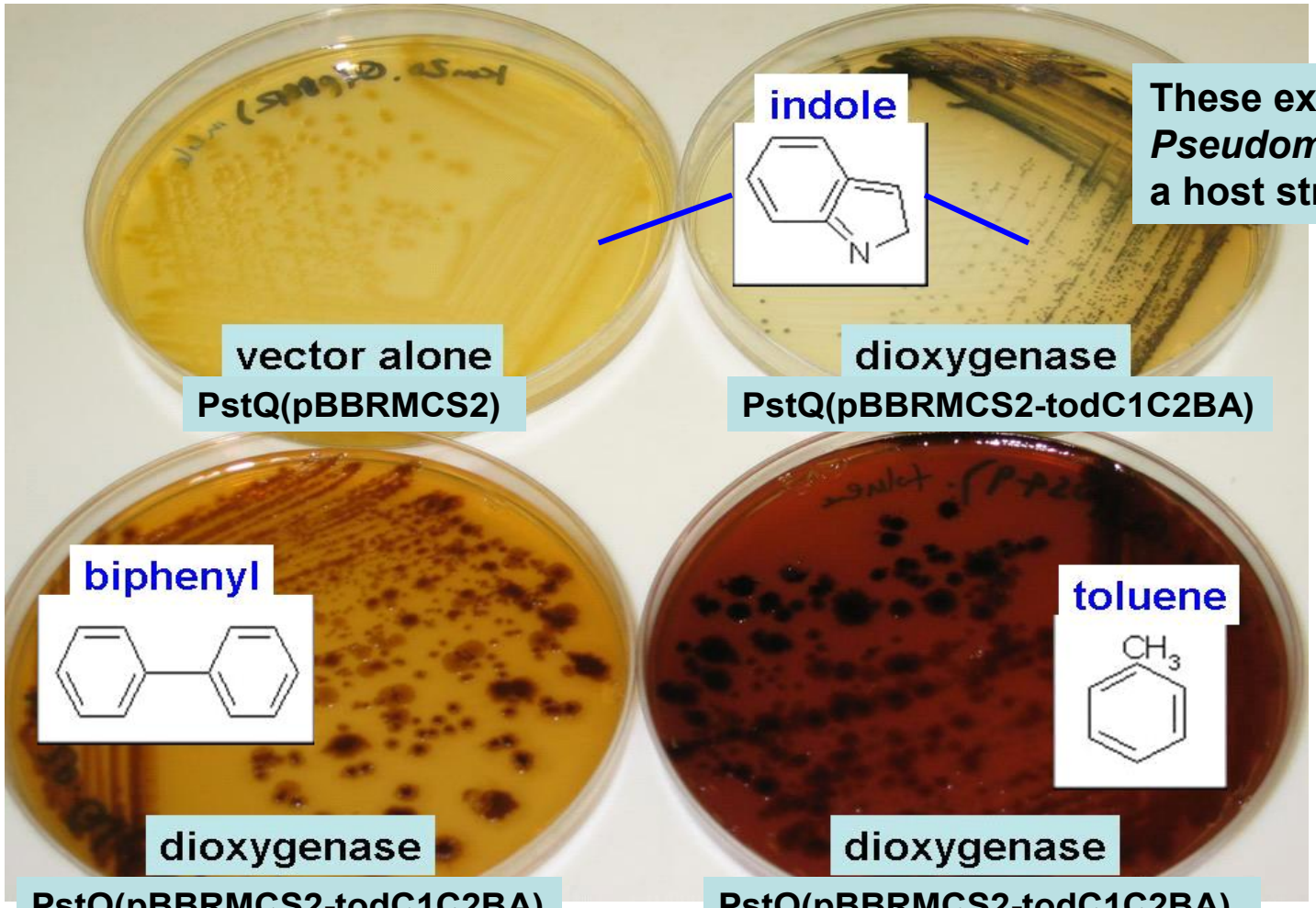


FIG. 4. **SDS-PAGE of extracts from clones that overexpress the *tod* gene products.** Extracts were prepared as described in the

we will use this method in MICR3052 prac

Toluene dioxygenase ... heterologous expression

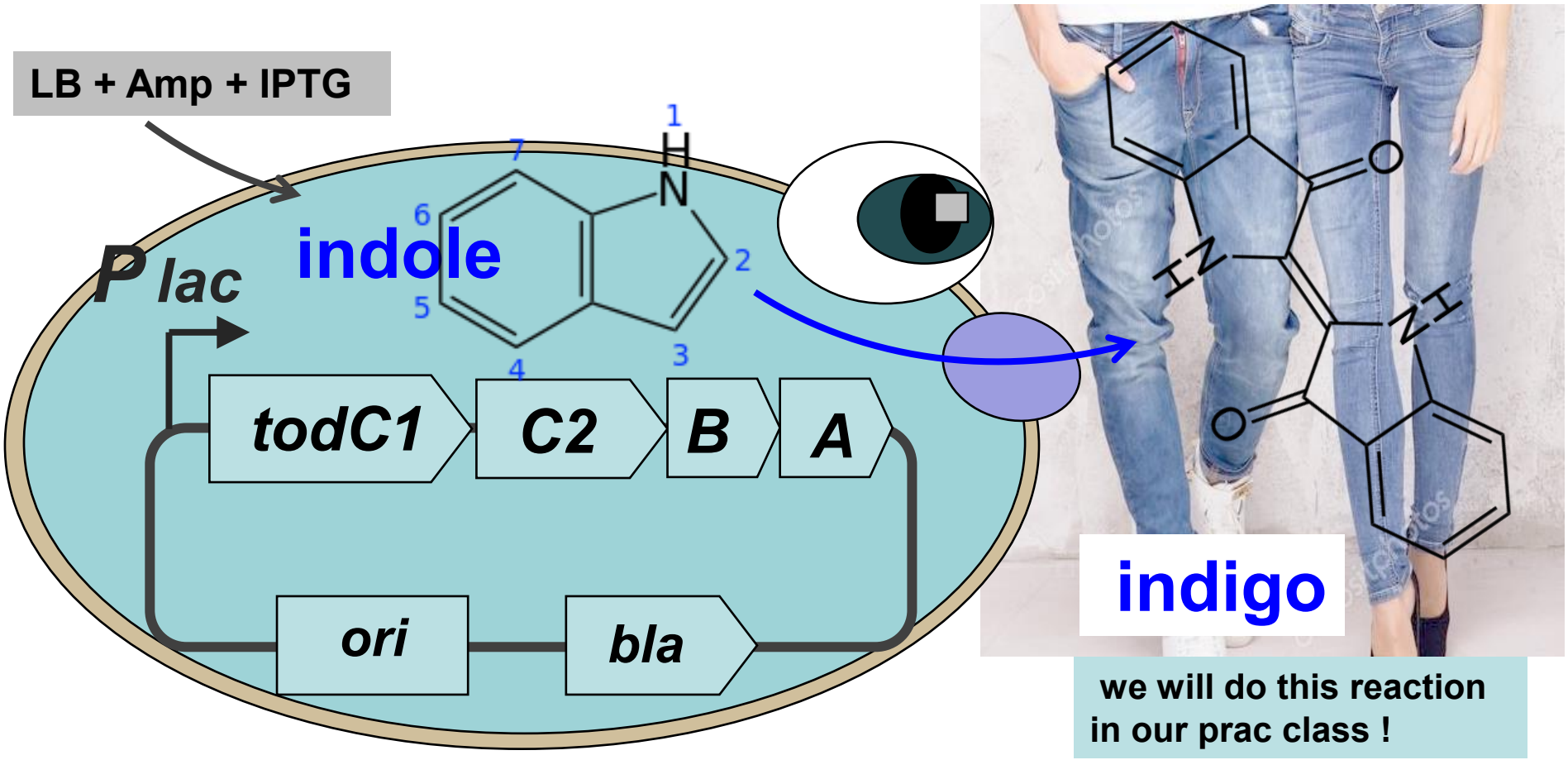
- TodC1C2BA attacks diverse substrates: toluene, biphenyl, indole
- colours here are the oxidation products: indigo, catechols



These experiments used *Pseudomonas stutzeri* as a host strain

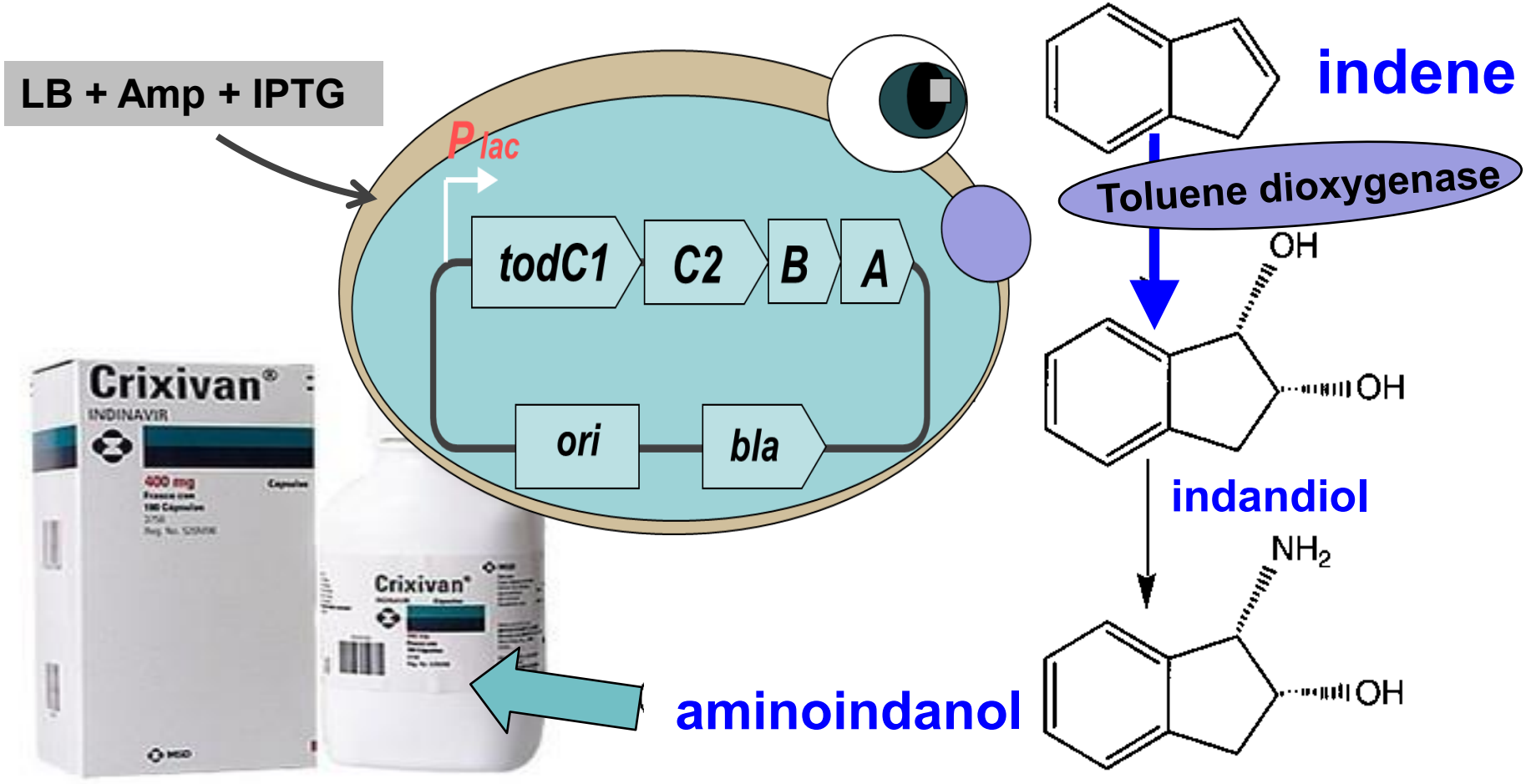
Toluene dioxygenase for biocatalysis 1.

- *E.coli* (*todC1C2BA*) oxidizes indole to indigo, a blue dye.
- Indole is native to E.coli, so synthesis needs no petrochemicals
- This is an example of cometabolism: host does not benefit



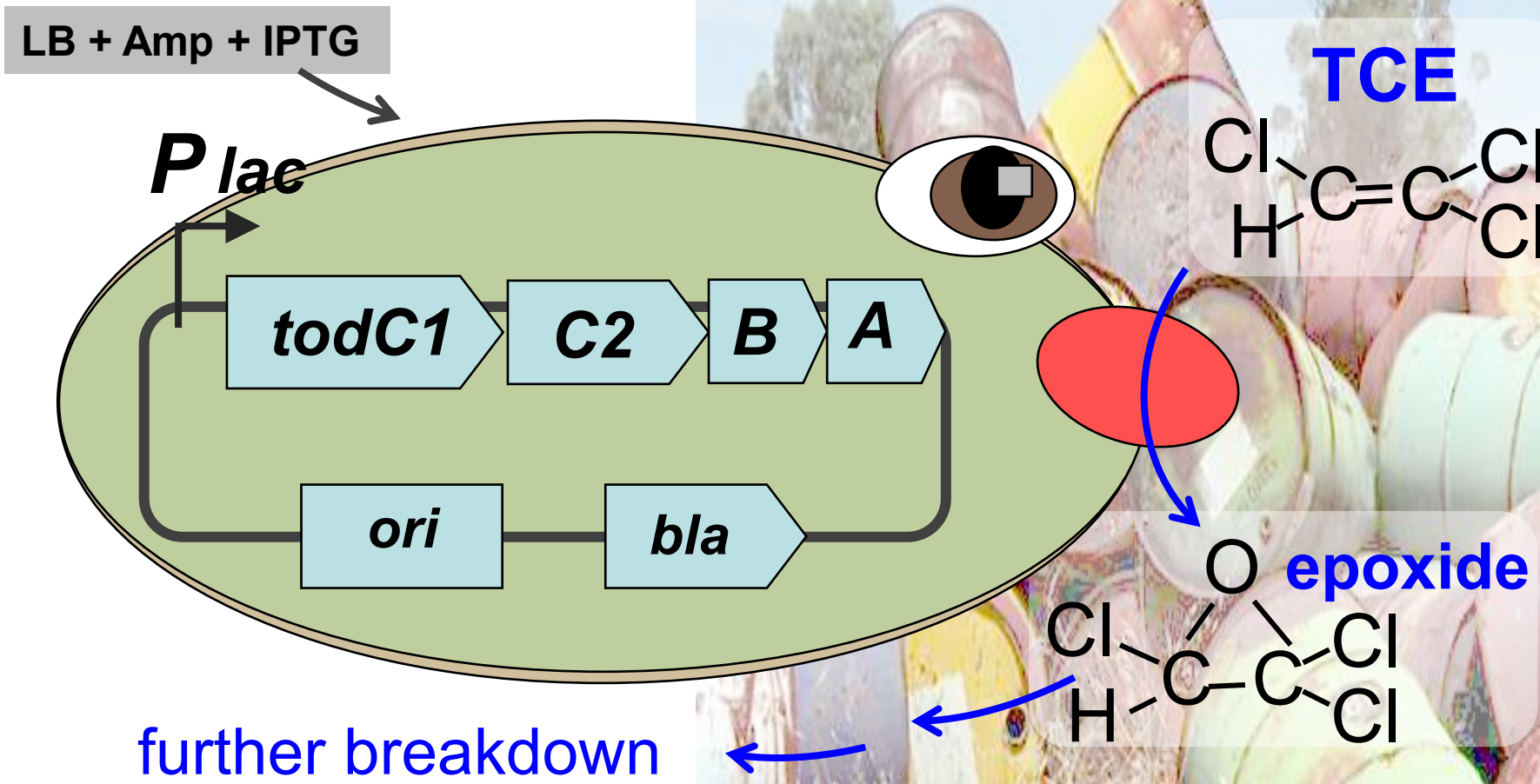
Toluene dioxygenase for biocatalysis 2.

- *E.coli* (*todC1C2BA*) oxidizes indene to indandiol, which is then converted to aminoindanol, a precursor for making antiviral drugs . Yes… cometabolism again



Toluene dioxygenase for breakdown of pollutants

- *E.coli* (*todC1C2BA*) cells are good at degrading TCE and other chlorinated alkanes and alkenes. Another example of cometabolism



Toluene dioxygenase for breakdown of pollutants

- Tol. diox. from PpF1 will attack TCE and many other pollutants
- This is important since cDCE and VC are made from TCE by anaerobic bacteria (see later section of this lecture)

TABLE 3. Substrate specificity displayed by *P. putida* F1 in the degradation of ethylene and chlorinated ethylenes

Substrate (20 μm initial concn)	% Decrease after 1 h[a]
Tetrachloroethylene	2
TCE	68
cis-1,2-Dichloroethylene	67
trans-1,2-Dichloroethylene	10
1,1-Dichloroethylene	18
Vinyl chloride	0
Ethylene	0

PPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1988, p. 1703–1708

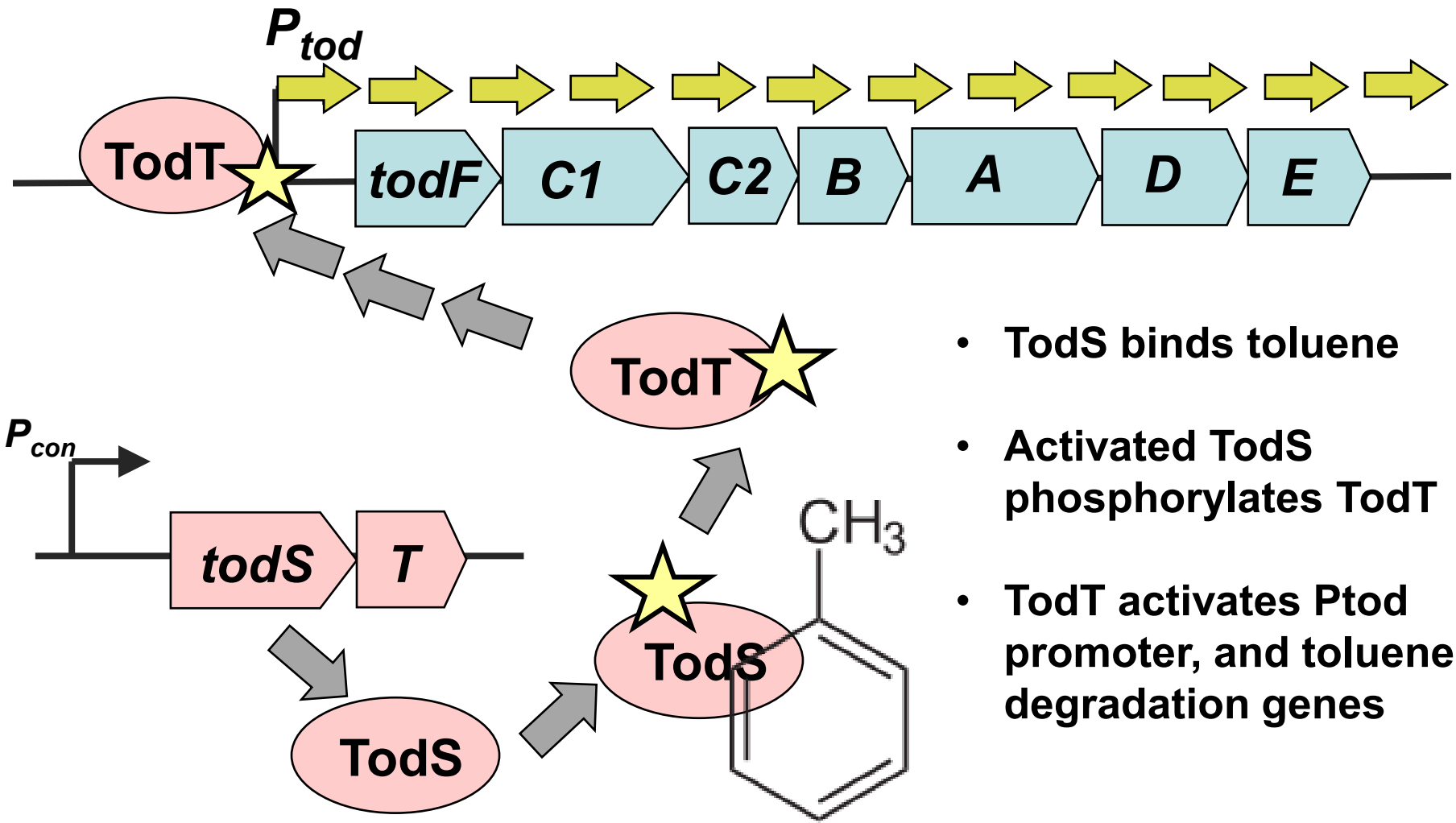
Degradation of Trichloroethylene by Toluene Dioxygenase in Whole-Cell Studies with *Pseudomonas putida* F1

LAWRENCE P. WACKETT[1]* AND DAVID T. GIBSON[2]

- Aerobic cometabolism via oxygenases is one major strategy for bioremediation of chlorinated pollutants

Regulation of toluene-degradation genes

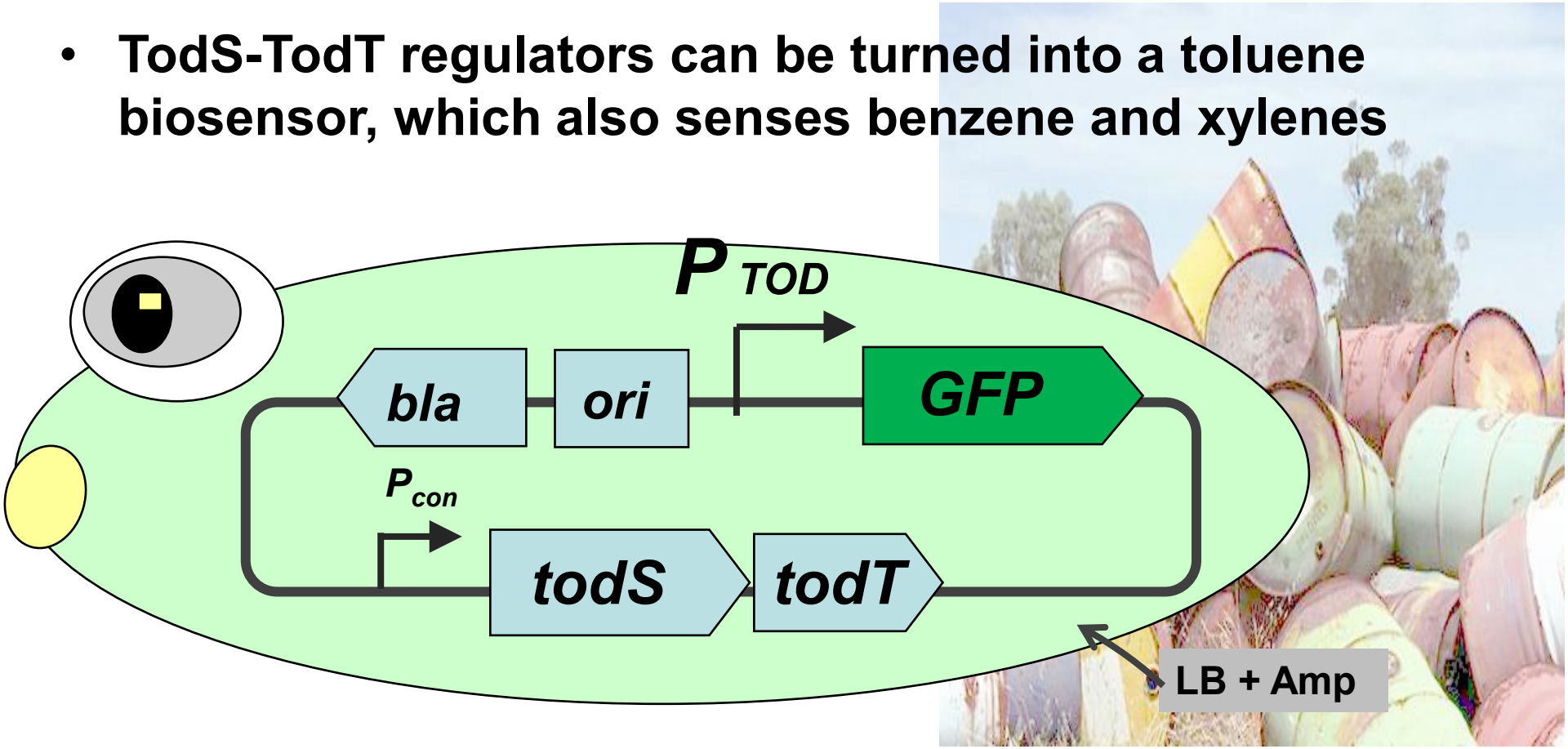
- Toluene degradation genes are organized as an operon in *Pseudomonas*, with a toluene-inducible promoter

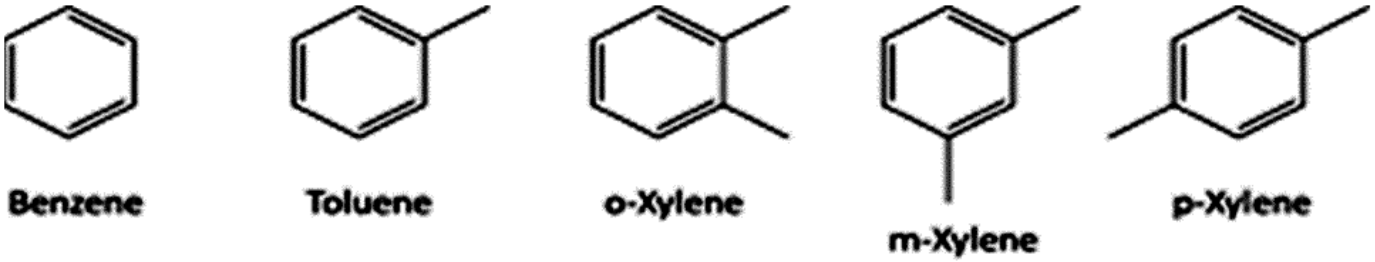


- TodS binds toluene
- Activated TodS phosphorylates TodT
- TodT activates Ptod promoter, and toluene degradation genes

Toluene regulator can be used as a biosensor

- TodS-TodT regulators can be turned into a toluene biosensor, which also senses benzene and xylenes

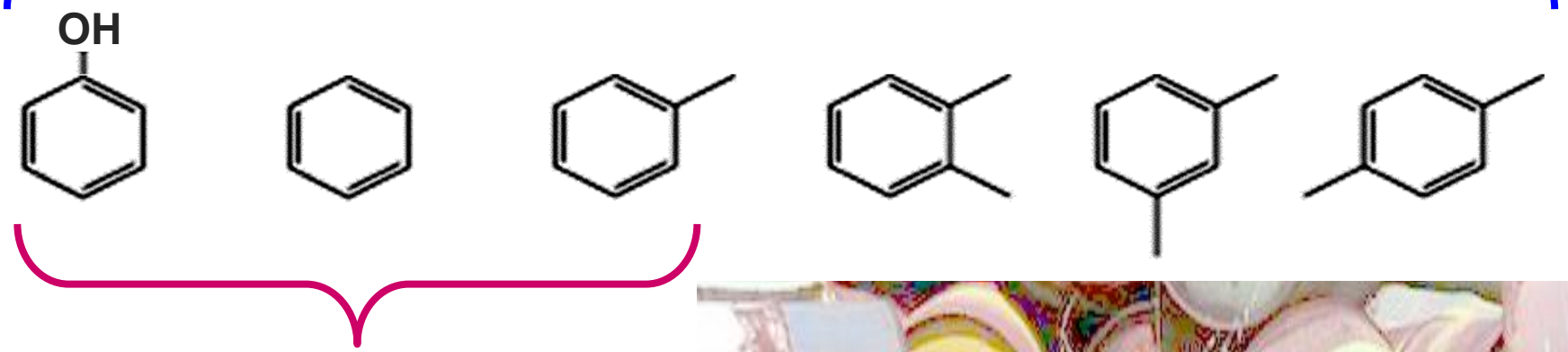




Substrate range – substrates vs. inducers

- Toluene is both an inducer of TodS and a substrate for the toluene dioxygenase in PpF1 but what about other compounds e.g. other aromatics?

All these aromatics are inducers of the toluene degradation system in PpF1



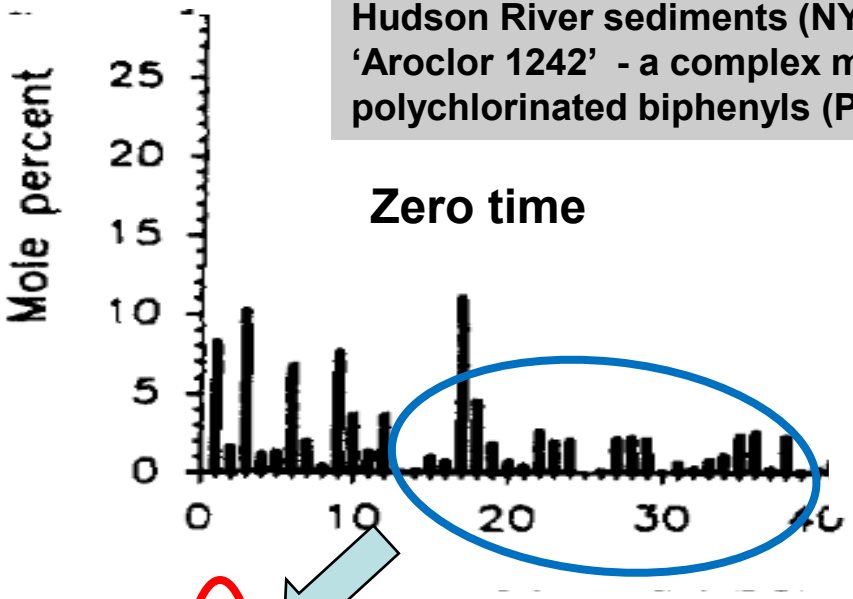
But only these three can support growth

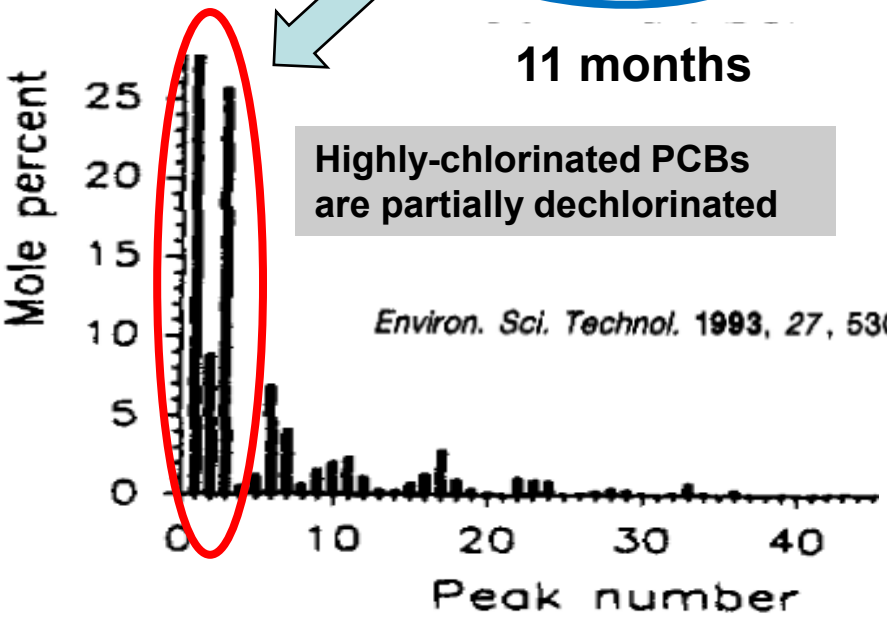
Part 2. Anaerobic biodegradation of chlorinated pollutants

Dehalococcoides and their dehalogenases

Biodegradation by anaerobic dechlorination – PCBs

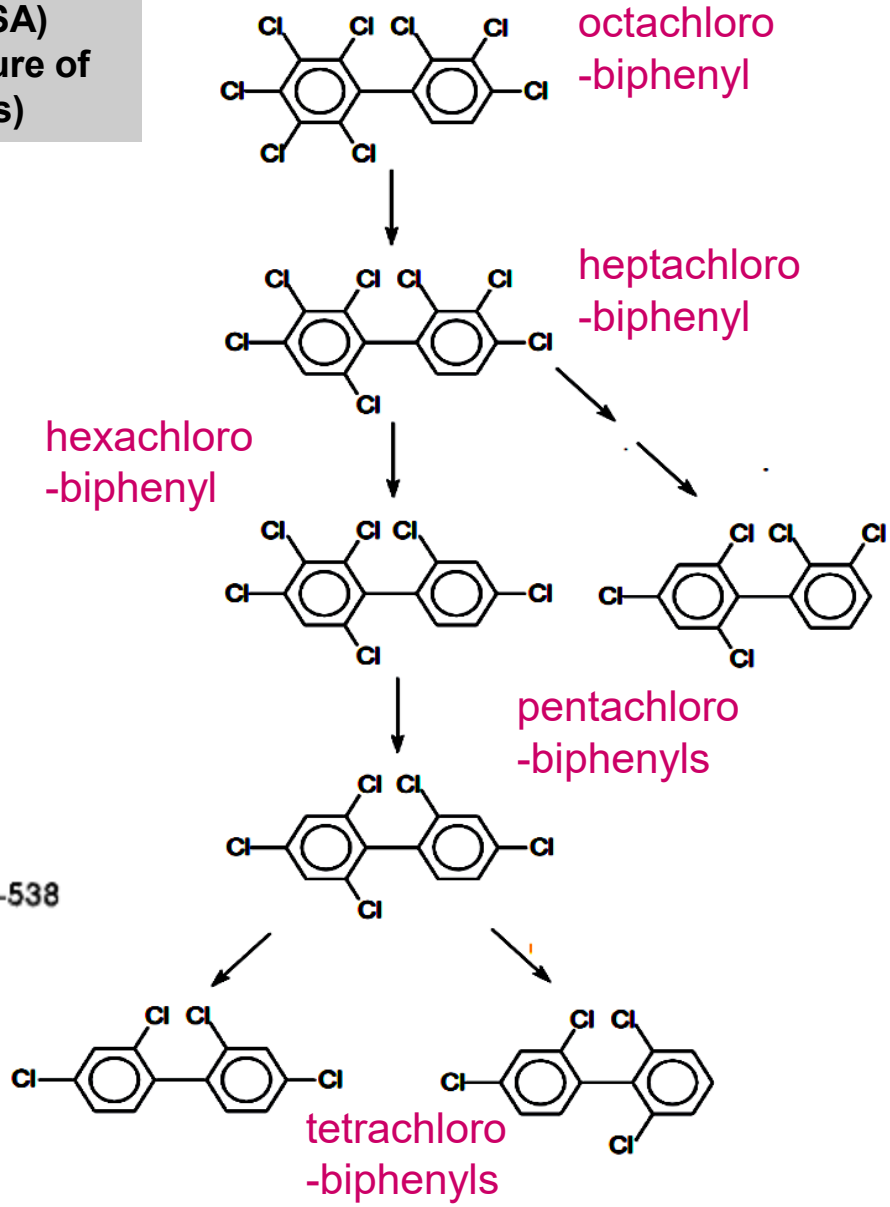
Hudson River sediments (NY, USA)
'Aroclor 1242' - a complex mixture of polychlorinated biphenyls (PCBs)





Highly-chlorinated PCBs are partially dechlorinated

Environ. Sci. Technol. **1993**, *27*, 530–538



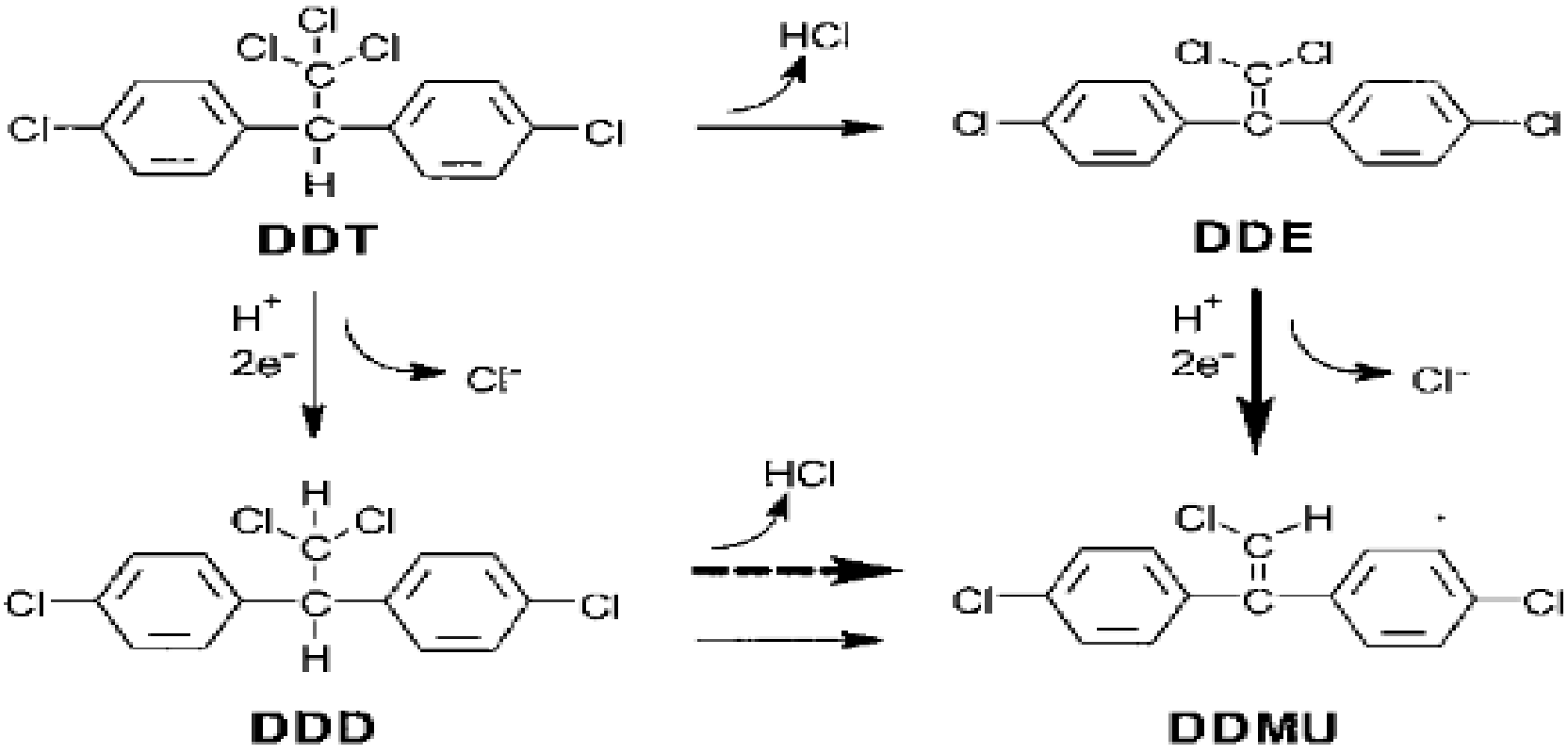
Biodegradation by anaerobic dechlorination – DDT

Reductive Dechlorination of DDE to DDMU in Marine Sediment Microcosms

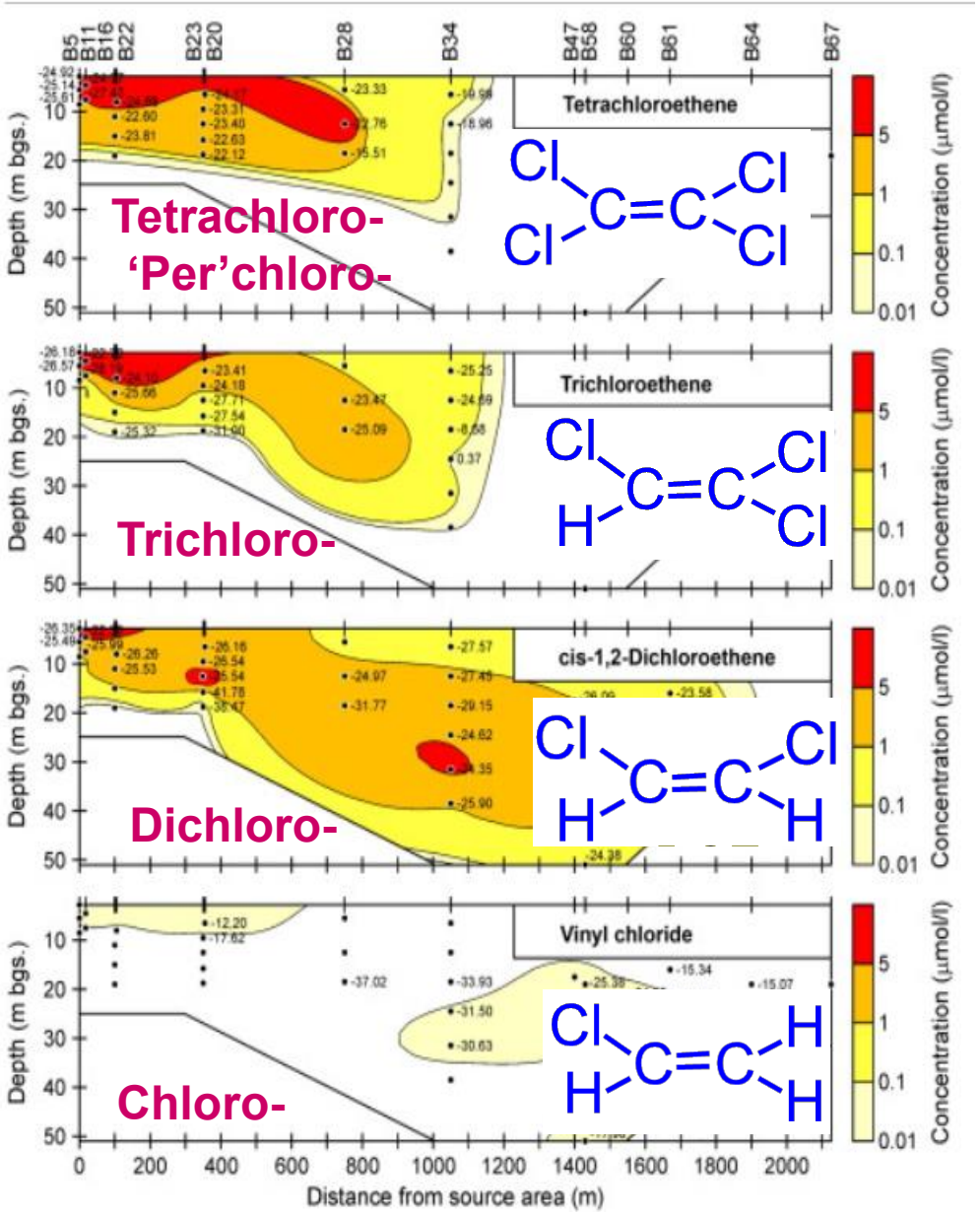
John F. Quensen III,* Sherry A. Mueller, Mahendra K. Jain, James M. Tiedje

Experiments with DDT-containing sediments show that DDE and DDD are dechlorinated to DDMU

SCIENCE • VOL. 280 • 1 MAY 1998 •



Biodegradation by anaerobic dechlorination – PCE / TCE



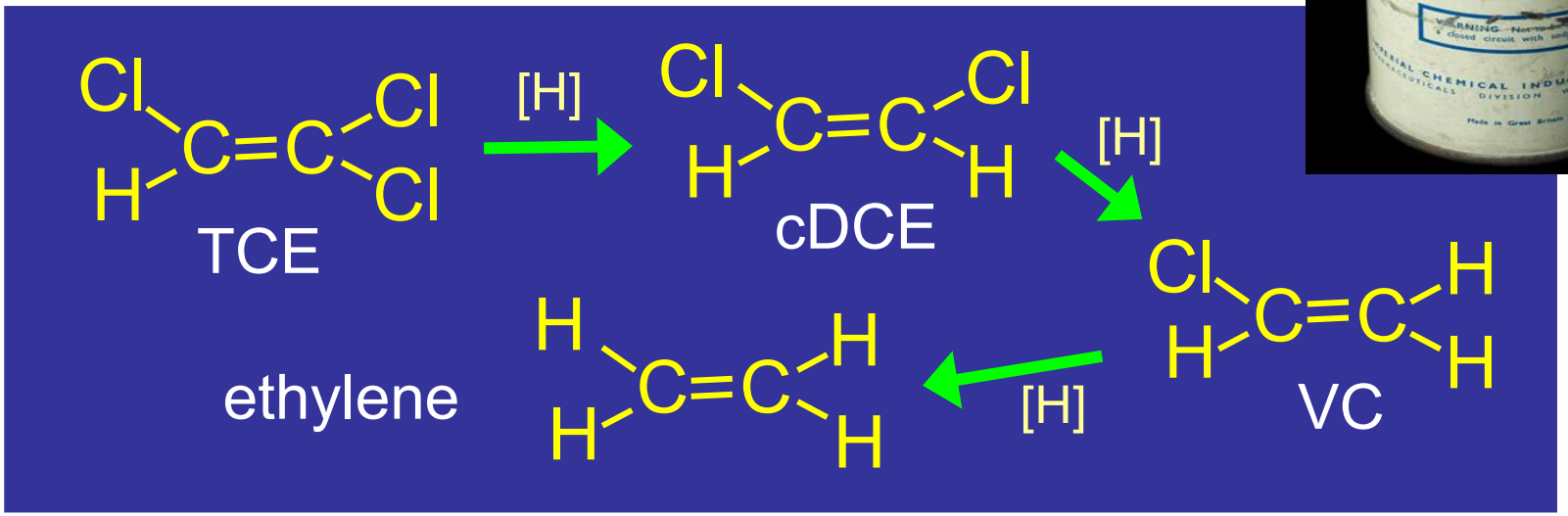
- PCE is primary contaminant in groundwater below a dry cleaner
- So why do we also find TCE, cDCE, and VC in this water?

→ these are the intermediates or end-products of PCE and TCE biodegradation by anaerobes

- This pattern is common at PCE- and TCE- contaminated sites

Biodegradation by anaerobic dechlorination – PCE / TCE

- **PCE & TCE: used as solvents, 'dry-cleaners', and anaesthetics. Common groundwater contaminants**
- **TCE is persistent in the environment but some bacteria can transform it**
- **TCE is REDUCED to ethylene**



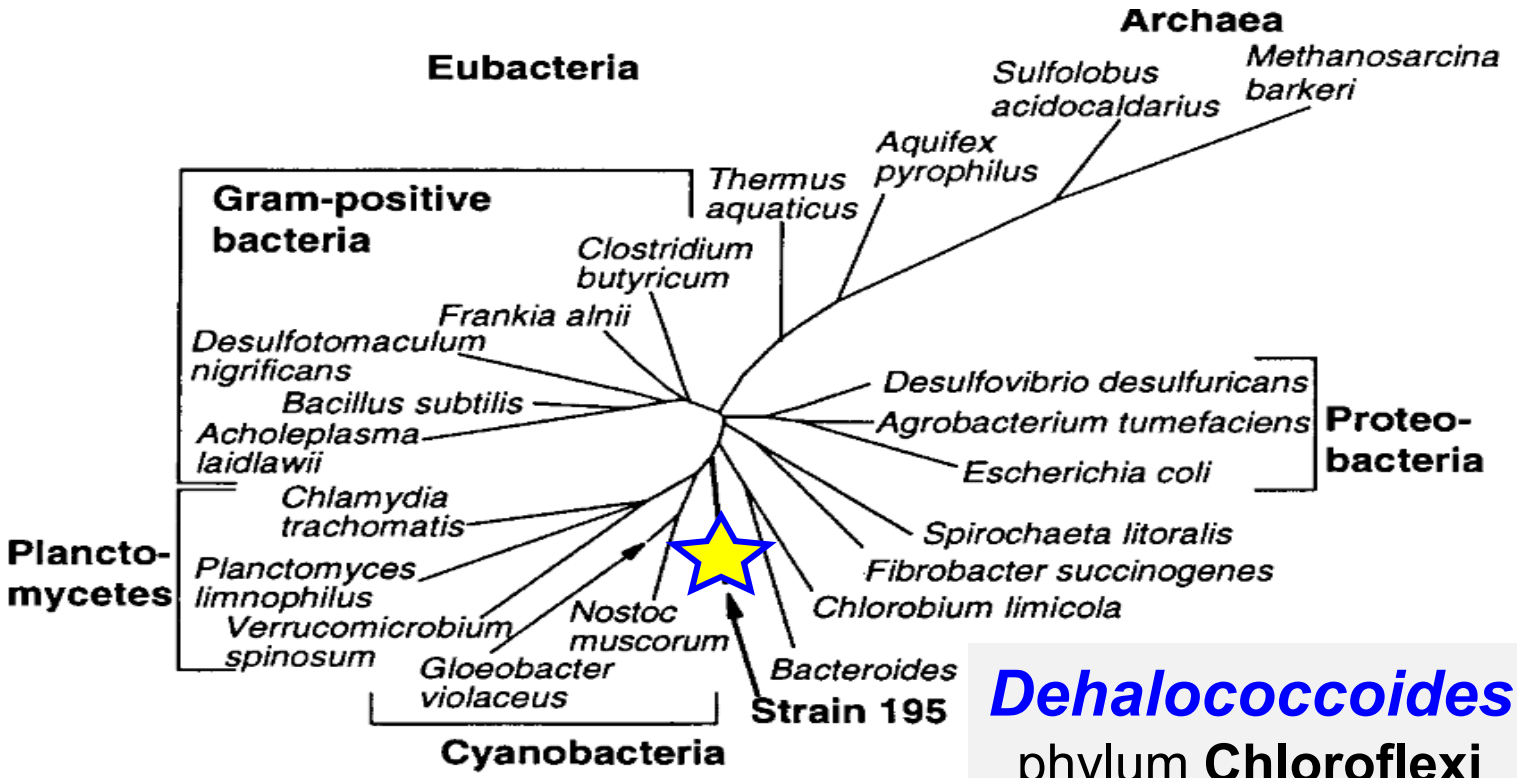
Dehalococcoides – a new genus of obligate organohalide-respiring bacteria

First identification of a pure culture of microbe that can grow on PCE/ TCE <u>as e- acceptors</u>

Isolation of a Bacterium That Reductively Dechlorinates Tetrachloroethene to Ethene

Xavier Maymó-Gatell, Yueh-tyng Chien, James M. Gossett, Stephen H. Zinder*

SCIENCE • VOL. 276 • 6 JUNE 1997



Dehalococcoides
phylum **Chloroflexi**

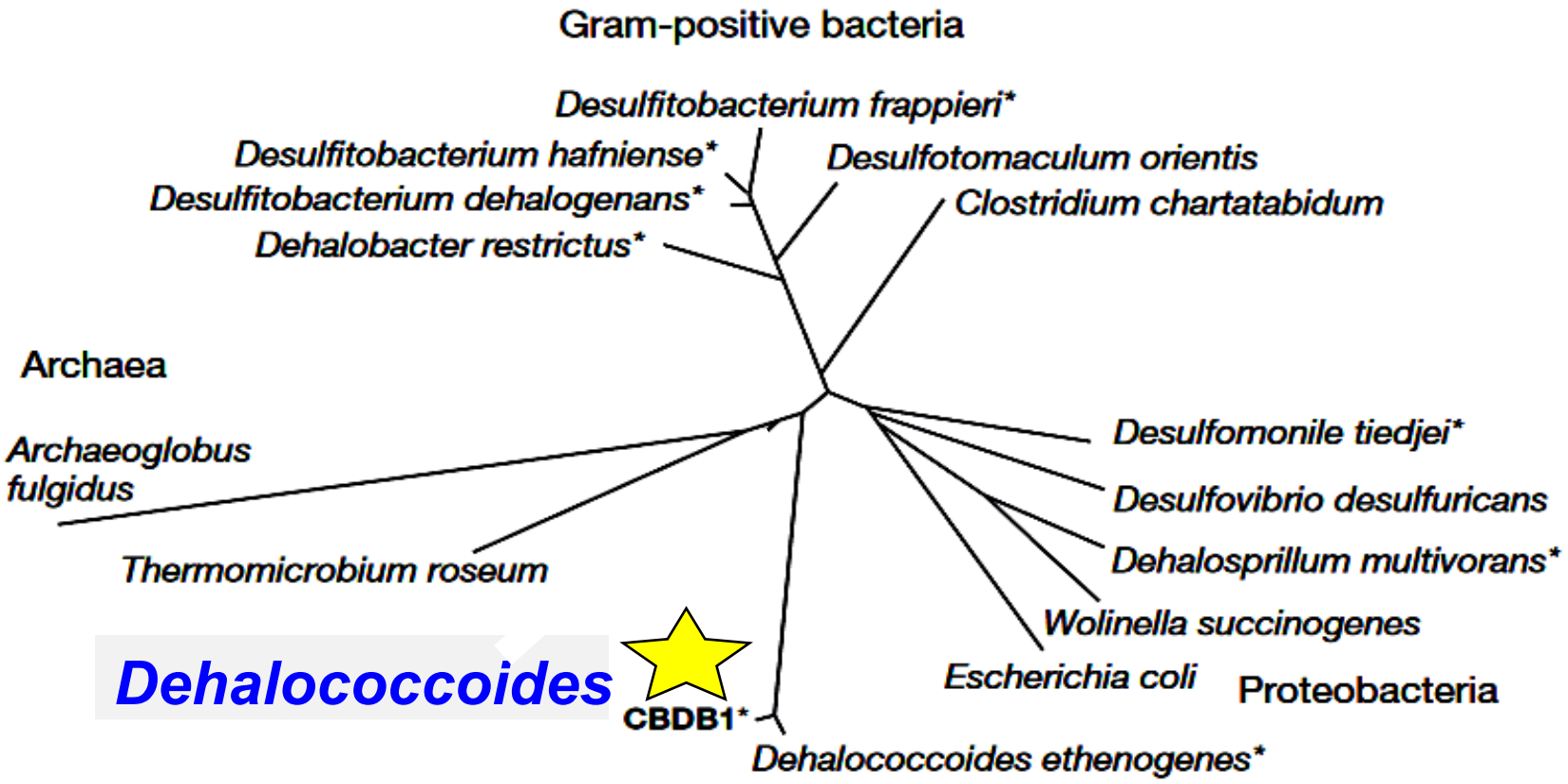
Dehalococcoides – a new genus of obligate organohalide-respiring bacteria

Same genus isolated again 3 years later as a chlorobenzene and PCB degrader

NATURE | VOL 408 | 30 NOVEMBER 2000 |

Bacterial dehalorespiration with chlorinated benzenes

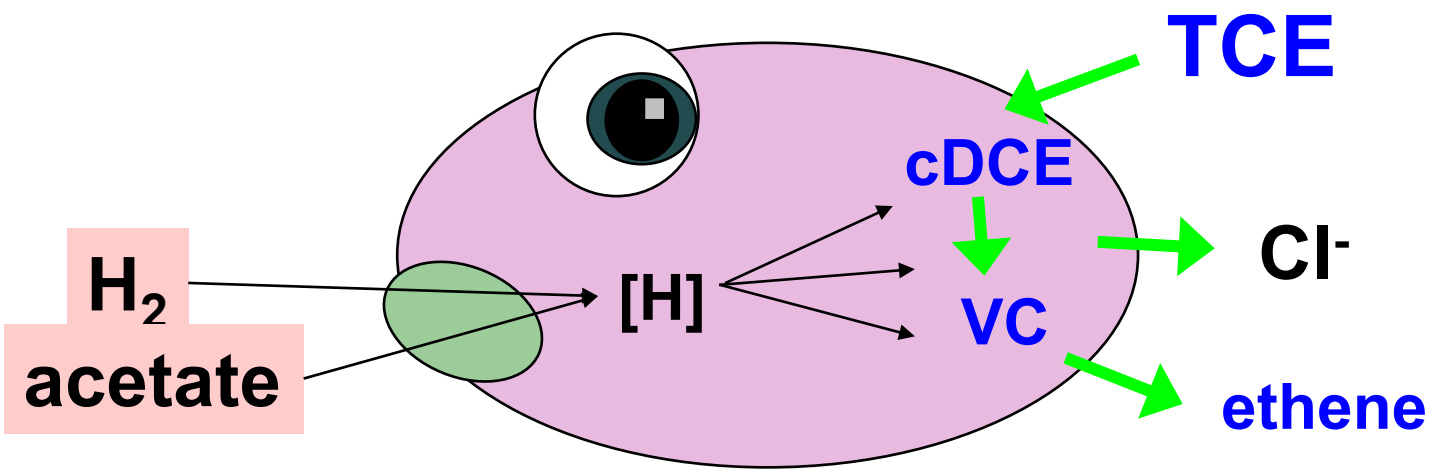
Lorenz Adrian*†, Ulrich Szewzyk†, Jörg Wecke‡ & Helmut Görisch*



Dehalorespiration – growth on chlorinated compounds as terminal electron acceptors

- This is a growth-linked process, cultures also need an electron donor (H_2) and a carbon source (e.g. acetate)

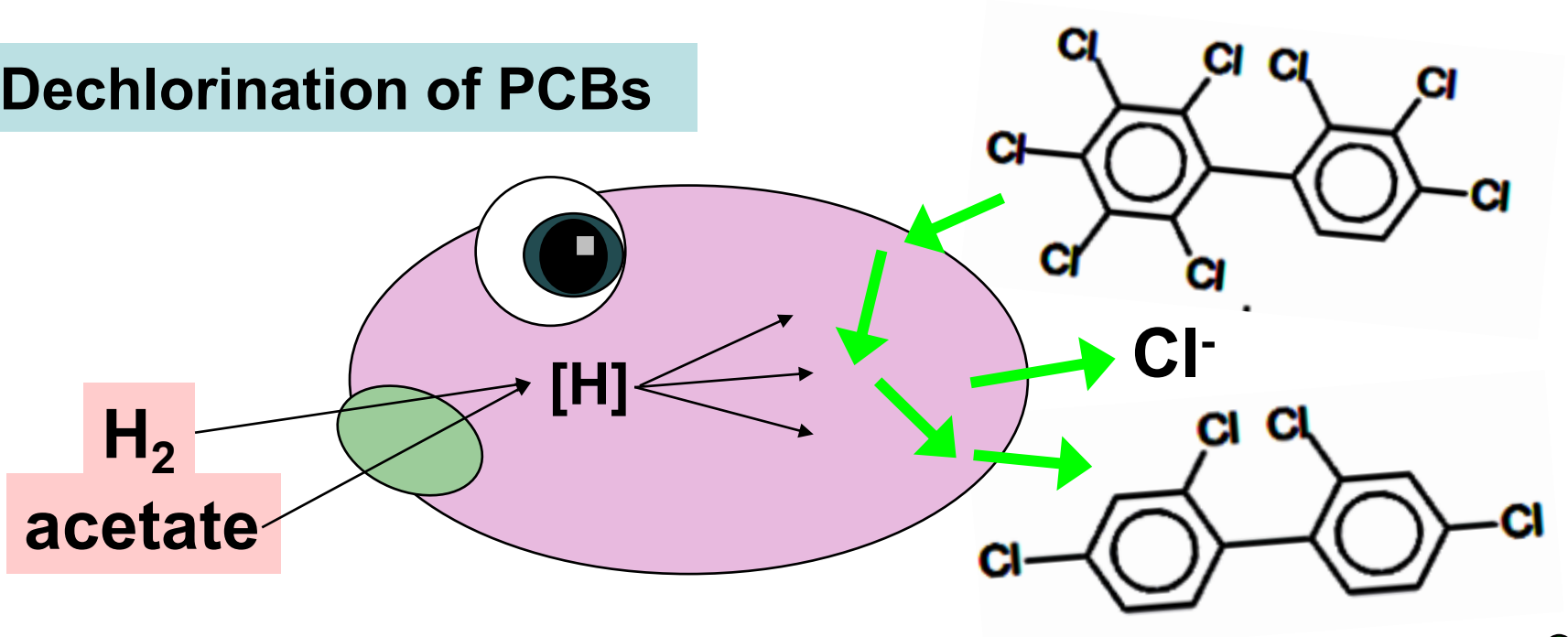
Dechlorination of TCE



- Anaerobic respiration via dehalogenases is a second major strategy for bioremediation of chlorinated pollutants

Dehalorespiration – growth on chlorinated compounds as terminal electron acceptors

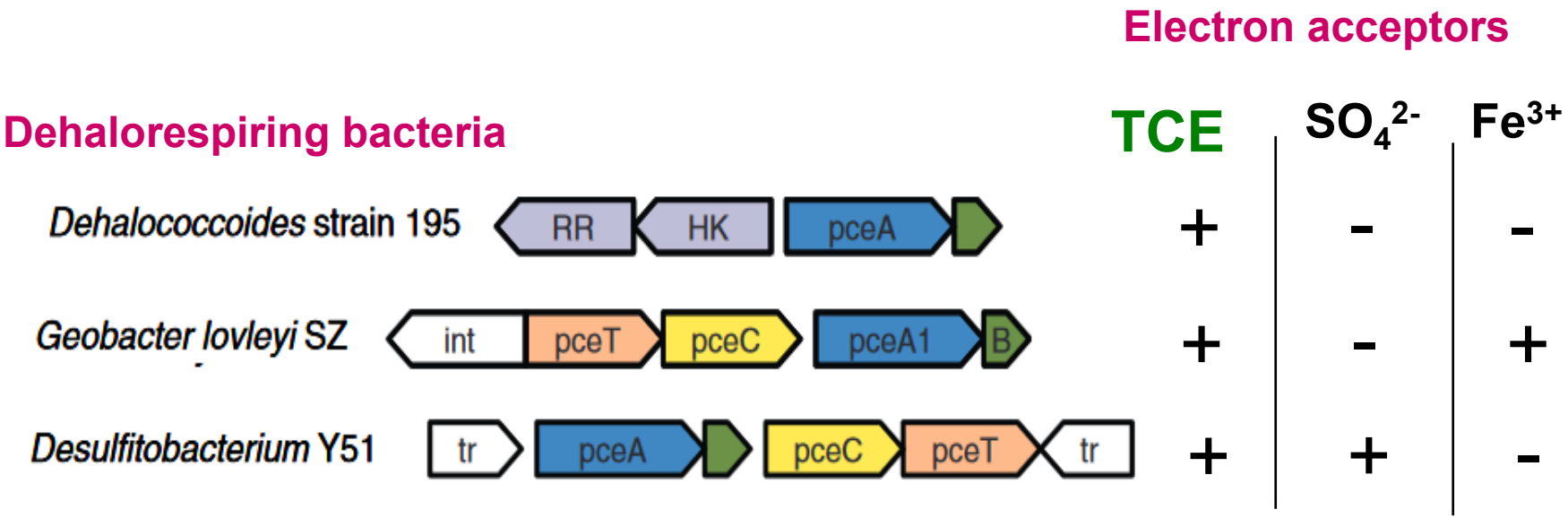
- This is a growth-linked process, cultures also need an electron donor (H_2) and a carbon source (e.g. acetate)



- Anaerobic respiration via dehalogenases is a second major strategy for bioremediation of chlorinated pollutants

Dehalorespiration – obligate vs facultative

- Some genera e.g. Dehalococcoides are 'obligate' dehalorespiring bacteria – can't use other e- acceptors
- Other genera are facultative e.g. Geobacter can also use iron while Desulfitobacterium can also use sulfate



Dehalorespiration – why so many dehalogenases?

- **Obligate dehalorespiring bacteria have <u>many</u> different dehalogenase genes in their genome vs. facultatives.**

<u>Why so many?</u> … and what are their 'natural' substrates?

Organism	Number of reductive dehalogenases	
Dehalococcoides ethenogenes 195	17	Obligate halorespirers
Dehalococcoides mccartyi BAV1	10	
Dehalococcoides mccartyi CBDB1	32	
Dehalococcoides mccartyi GT	20	
Desulfitobacterium dehalogenans	6	Facultative halorespirers
Desulfitobacterium dichloroeliminans LMG P-21439	1	
Desulfitobacterium hafniense DCB-2	7	
Desulfitobacterium sp. PCE1, DSM 10344	6	

Dehalorespiration – what are the *natural* substrates for reductive dehalogenases ?

- **Halogenated compounds are found in nature, if you look for them. e.g. algae make haloalkenes similar to TCE**

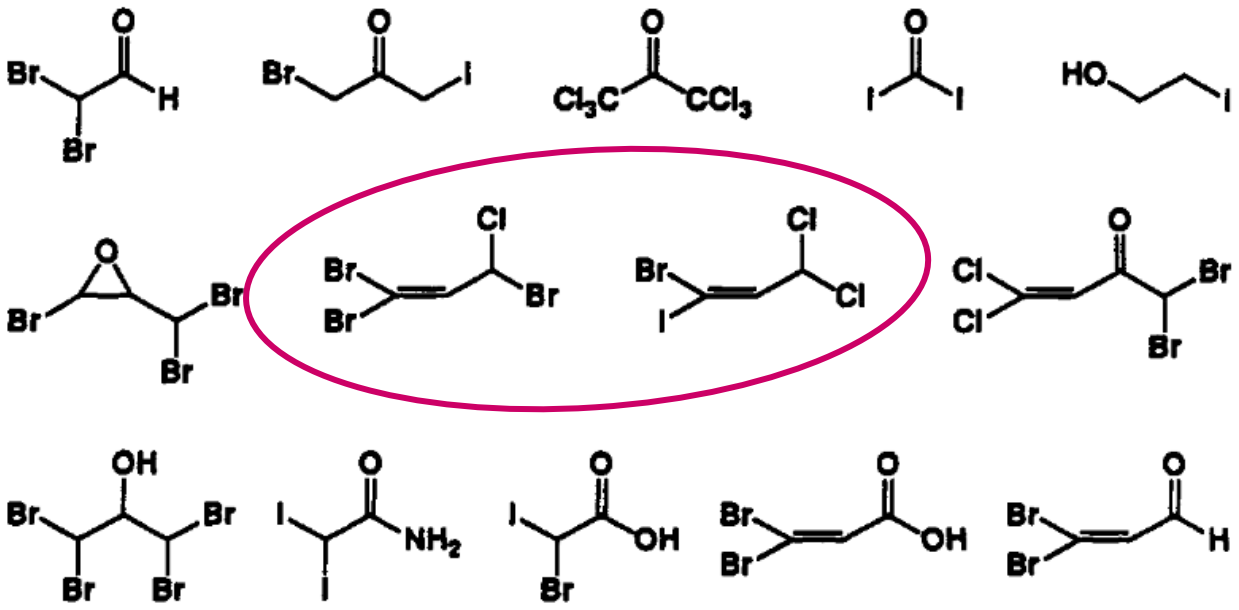


FIGURE 1. Representative compounds produced by the marine alga *Asparagopsis taxiformis* (35).

Journal of Natural Products
Vol. 55, No. 10, pp. 1353-1395, October 1992