PHYSIOLOGICAL AND ENZYMATIC DIVERSITY OF AEROBIC MTBE BIODEGRADATION PROCESSES

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API

Outline

- **MTBE biodegradation: Our interests and approaches**
- ***Rubrivivax gelatinosa* PM1**
- ***Mycobacterium vaccae* JOB5**
- **N-alkane-oxidizing *Pseudomonas* strains**
- **Conclusions and potential environmental significance**

Our Interests

- We are interested in determining the diversity of the pathways of aerobic MTBE and TBA oxidation (metabolism *vs* cometabolism)
- We want to identify the enzymes responsible for initiating these processes at the molecular level
- We want to understand how distinct microbial processes can contribute to MTBE (and TBA) degradation in gasoline-impacted environments

Our Approaches

- **Growth experiments:**
  - Batch culturing organisms in mineral salts medium with individual substrates
  - Measurement of culture density (OD_{600})
- **Degradation experiments:**
  - ~200-fold concentrate of cells grown on individual substrates
  - Small scale reactions (1-10ml) with MTBE, TBA *etc*. typically added individually
  - Substrate/product detected by DAI-FID GC

MTBE Metabolism and Cometabolism
A Biodegradation Continuum

full metabolism	slow metabolism	cometabolism
mineralization	incomplete oxidation	partial oxidation

Rubrivivax PM-1	***Mycobacterium sp.***	***Pseudomonas sp.***
Hydrogenophaga flava	***Arthrobacter sp.***	**Many others**
Slow growth on MTBE and TBA	No (?) growth on MTBE and TBA	No growth on MTBE and TBA
TBA intermediate	TBA intermediate	TBA intermediate
TBA oxidized	TBA oxidized	TBA not oxidized
	TBF intermediate	

Rubrivivax gelatinosa PM1

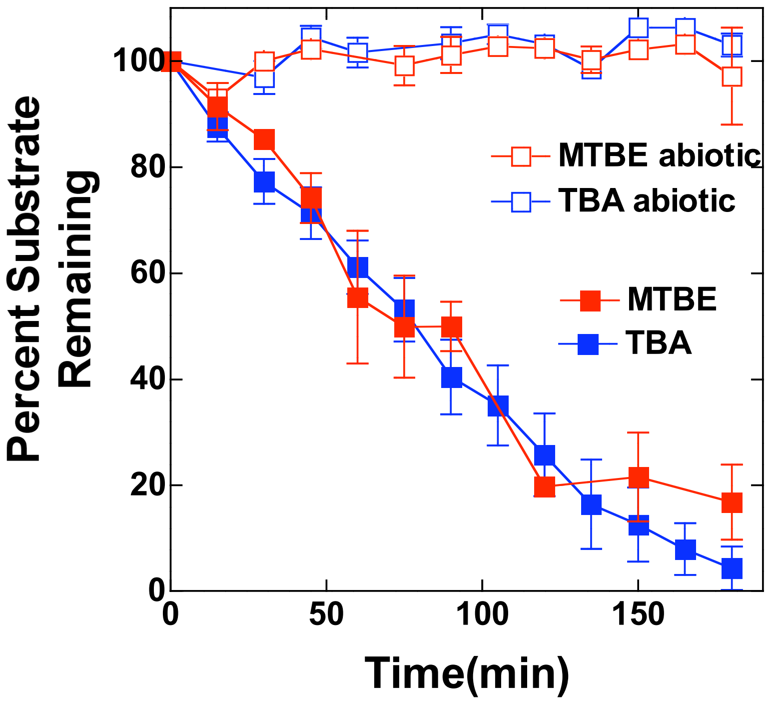
- Originally isolated from peat moss biofilter for MTBE/gasoline-impacted ground water.
- One of very limited number of organisms currently known to grow on MTBE as sole C and energy source.
- PM1 or “PM1-like” organisms have been shown by molecular analysis to be present during MTBE biodegradation in field and lab scale studies
- Little is currently known about either the pathway or enzymes involved in MTBE oxidation in this organism (even though genome has now been sequenced)

PM1 Growth Substrate Range

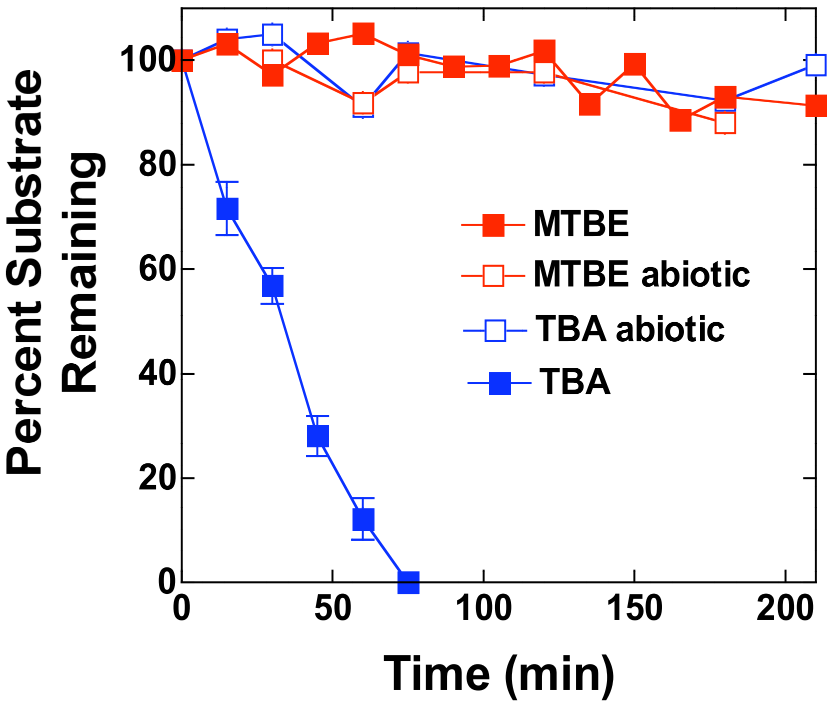
Potential Growth Substrate	Absorbance (600 nm) after 12 d.	Potential Growth Substrate	Absorbance (600 nm) after 12 d.
n-Alkanes:		**Aromatics:**	
methane	0.009 (0.002)	benzene	0.320 (0.008)
ethane	0.005 (0.001)	toluene	0.001 (≤0.001)
propane	0.009 (0.003)	ethylbenzene	0.001 (≤0.001)
n-butane	0.006 (0.003)	*o*-xylene	0.001 (≤0.001)
n-pentane	0.001 (≤0.001)	*m*-xylene	0.001 (≤0.001)
n-hexane	0.001 (≤0.001)	*p*-xylene	0.001 (≤0.001)
n-heptane	0.001 (≤0.001)	**Oxygenates & Ethers**	
n-octane	0.001 (≤0.001)	MTBE	0.199 (0.005)
1º Alcohols		ETBE	0.002 (0.001)
methanol	0.044 (0.002)	TBA	0.210 (0.005)
ethanol	0.546 (0.010)	2M12PD (MPD)	0.255 (0.011)
1-propanol	0.590 (0.007)	2M13PD	0.264 (0.006)
1-butanol	0.677 (0.018)	2M1P	0.035 (0.001)
1-pentanol	0.694 (0.035)	TAME	0.003 (0.001)
1- heptanol	0.529 (0.011)	TAA	0.006 (0.003)
1- octanol	0.001 (≤0.001)	DIPE	0.006 (0.002)
2º Alcohols:		butyl methyl ether	0.007 (0.002)
2-propanol	0.255 (0.048)	*sec*-butyl methyl ether	0.006 (0.002)
2-butanol	0.023 (0.015)	ethyl ether	0.006 (0.001)
2-pentanol	0.012 (0.007)	**Others**	
2-hexanol	0.017 (0.004)	formate	0.001 (≤0.001)
2-heptanol	0.020 (0.01)	formaldehyde	0.019 (0.002)
ketones		methacrylic acid	0.283 (0.013)
acetone	0.218 (0.002)	methylacetate	0.001 (≤0.001)
hydroxyacetone	0.05 (0.014)	isobutane	0.036 (0.003)
2-butanone	0.001 (≤0.001)	isobutylene	0.018 (0.004)
2-pentanone	0.001 (≤0.001)		

Rubrivivax PM1-
Two Enzymes for MTBE and TBA

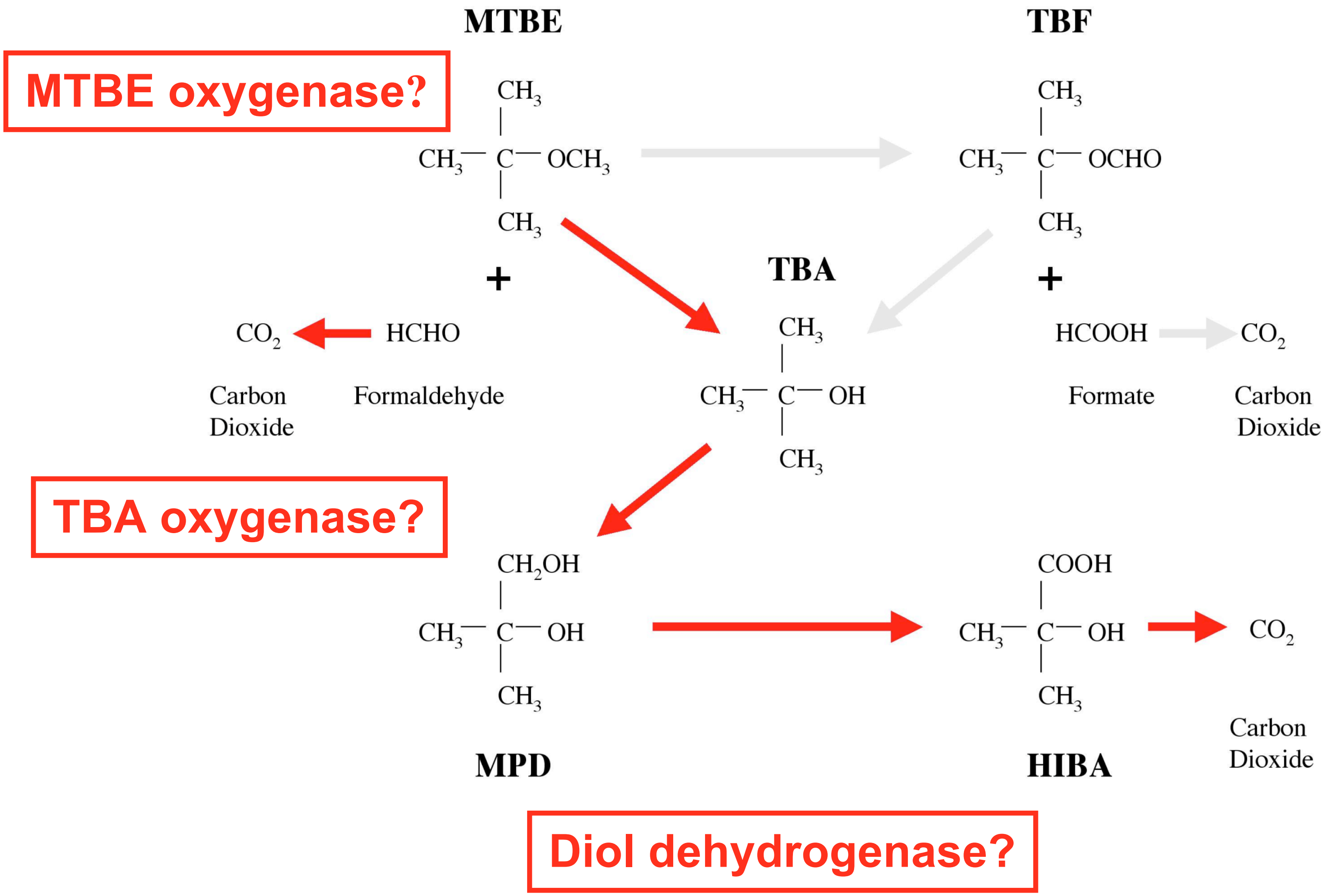
MTBE and TBA Oxidation by MTBE-Grown Cells



MTBE and TBA Oxidation by TBA-Grown Cells



PM1 Pathway and Enzymes



MTBE oxygenase?

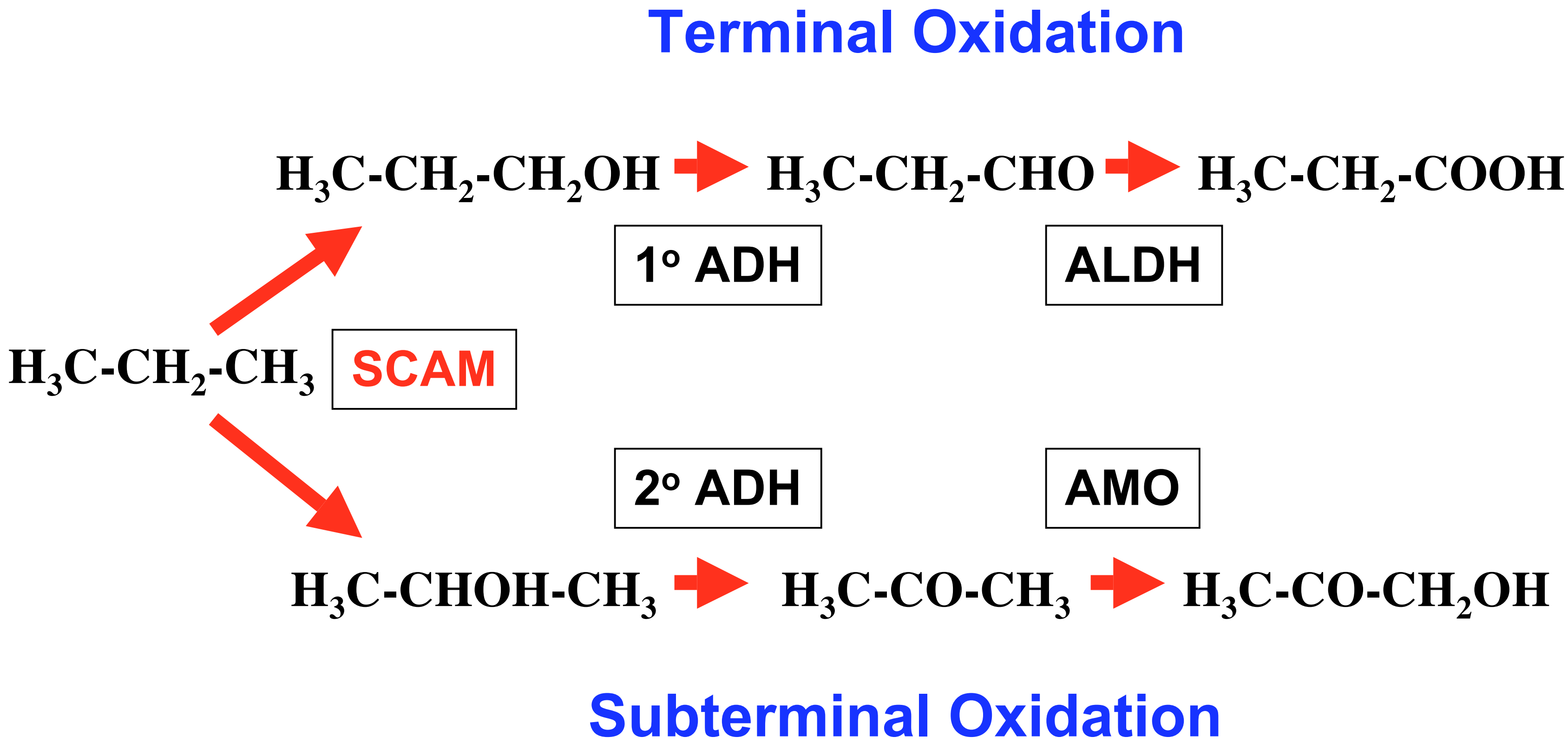
TBA oxygenase?

Diol dehydrogenase?

Mycobacterium vaccae JOB5

- Metabolically diverse soil bacterium isolated from gasoline-impacted soils (1963 Foster)
- Only commercially strain enriched and isolated using branched alkane (isopentane)
- Grows on C_2-C_{22}-n-alkanes and branched alkanes
- Grows on aromatics (toluene and xylenes)
- Cells grown on short-chain n-alkanes (C_2-C_8) cometabolically oxidize wide range of pollutants:
  - **MTBE, TBA, 14D, THF, TCE, EDB *etc. etc.***
- aka: *Mycobacterium austroafricanum*

Initial Reactions in Propane Oxidation by *M. vaccae* JOB5



Terminal Oxidation

$H_3C\text{-}CH_2\text{-}CH_2OH \rightarrow H_3C\text{-}CH_2\text{-}CHO \rightarrow H_3C\text{-}CH_2\text{-}COOH$

1° ADH

ALDH

$H_3C\text{-}CH_2\text{-}CH_3$

SCAM

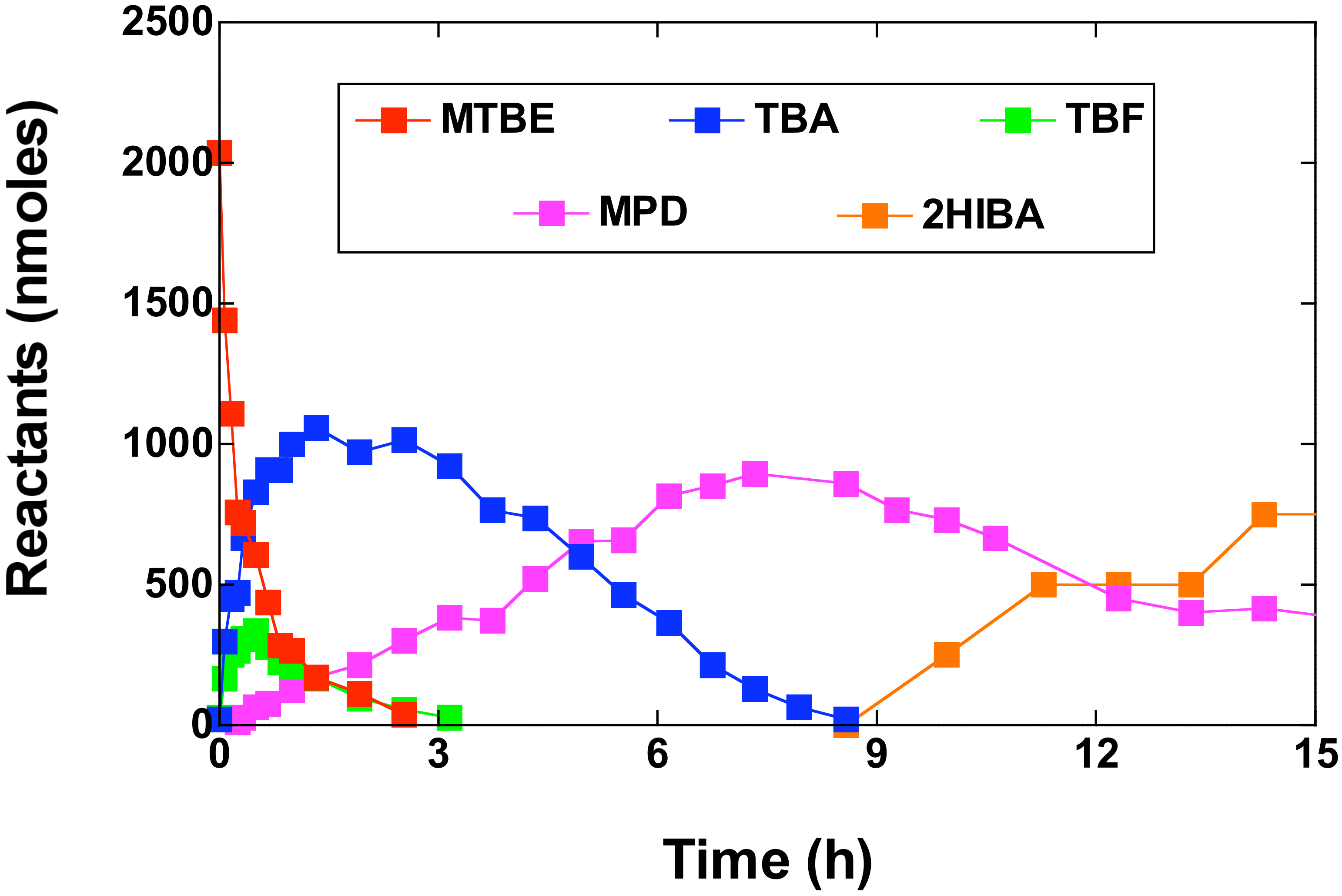
2° ADH

AMO

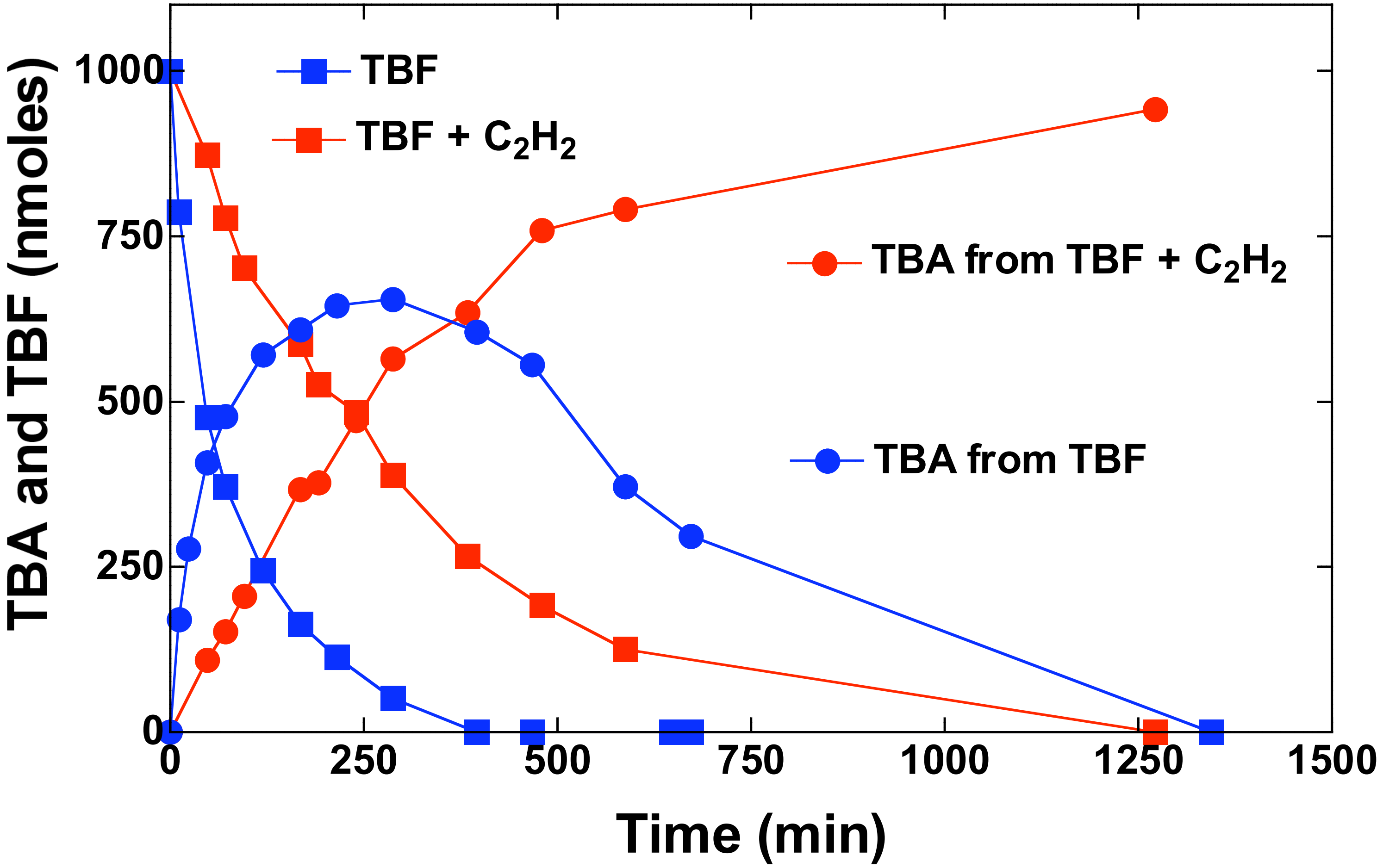
$H_3C\text{-}CHOH\text{-}CH_3 \rightarrow H_3C\text{-}CO\text{-}CH_3 \rightarrow H_3C\text{-}CO\text{-}CH_2OH$

Subterminal Oxidation

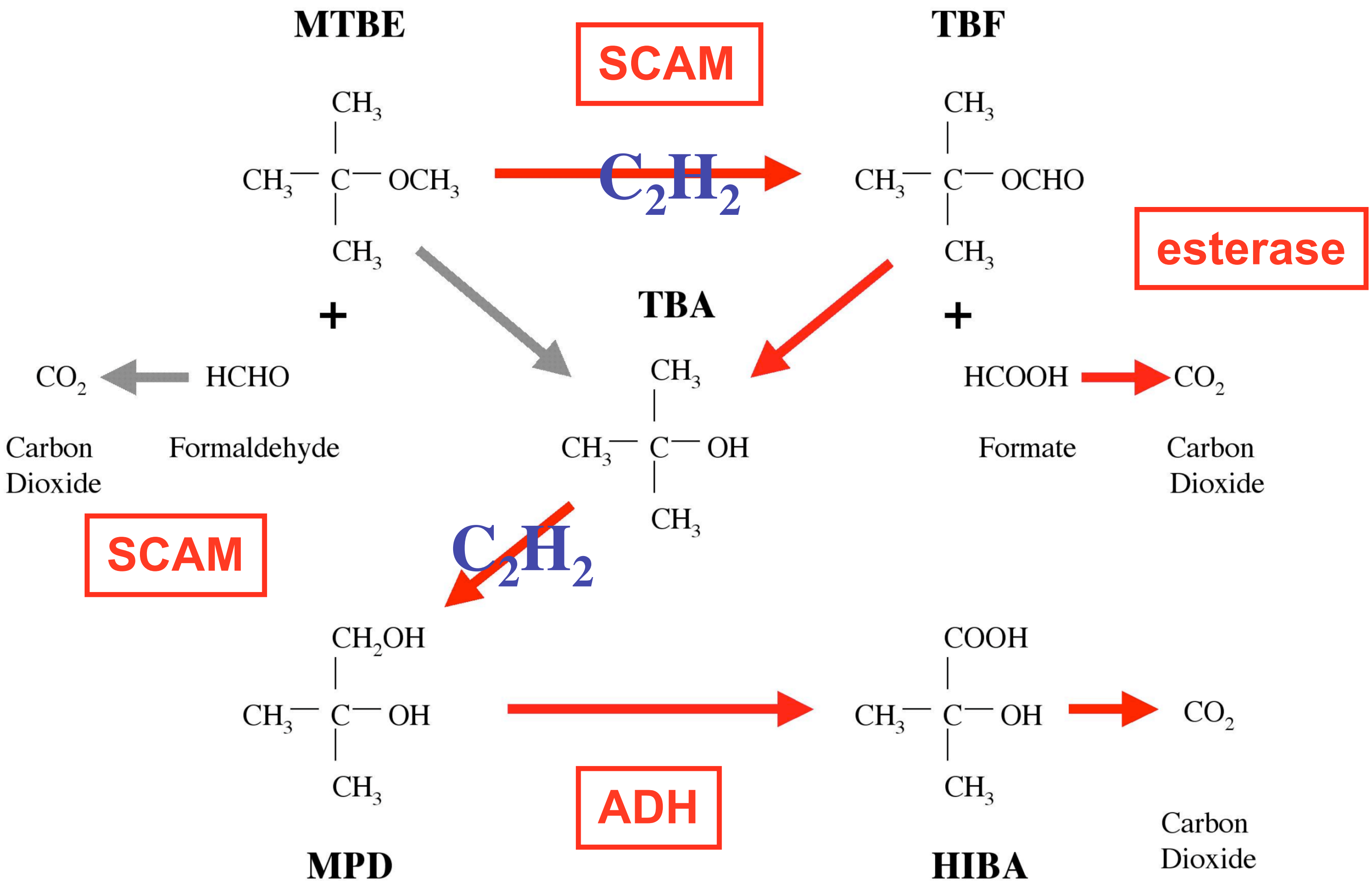
MTBE and TBA Degradation by Propane-Grown *M. vaccae* JOB5



Effect of C_2H_2 on TBF Hydrolysis and TBA Oxidation by *M. vaccae*



M. vaccae Pathway and Enzymes



MTBE

$CH_3-C(CH_3)_2-OCH_3$

SCAM

C_2H_2

TBF

$CH_3-C(CH_3)_2-OCHO$

+

$HCOOH \rightarrow CO_2$

Formate

Carbon Dioxide

esterase

+

$CO_2 \leftarrow HCHO$

Carbon Dioxide

Formaldehyde

TBA

$CH_3-C(CH_3)_2-OH$

SCAM

C_2H_2

MPD

$CH_3-C(CH_3)(CH_2OH)-OH$

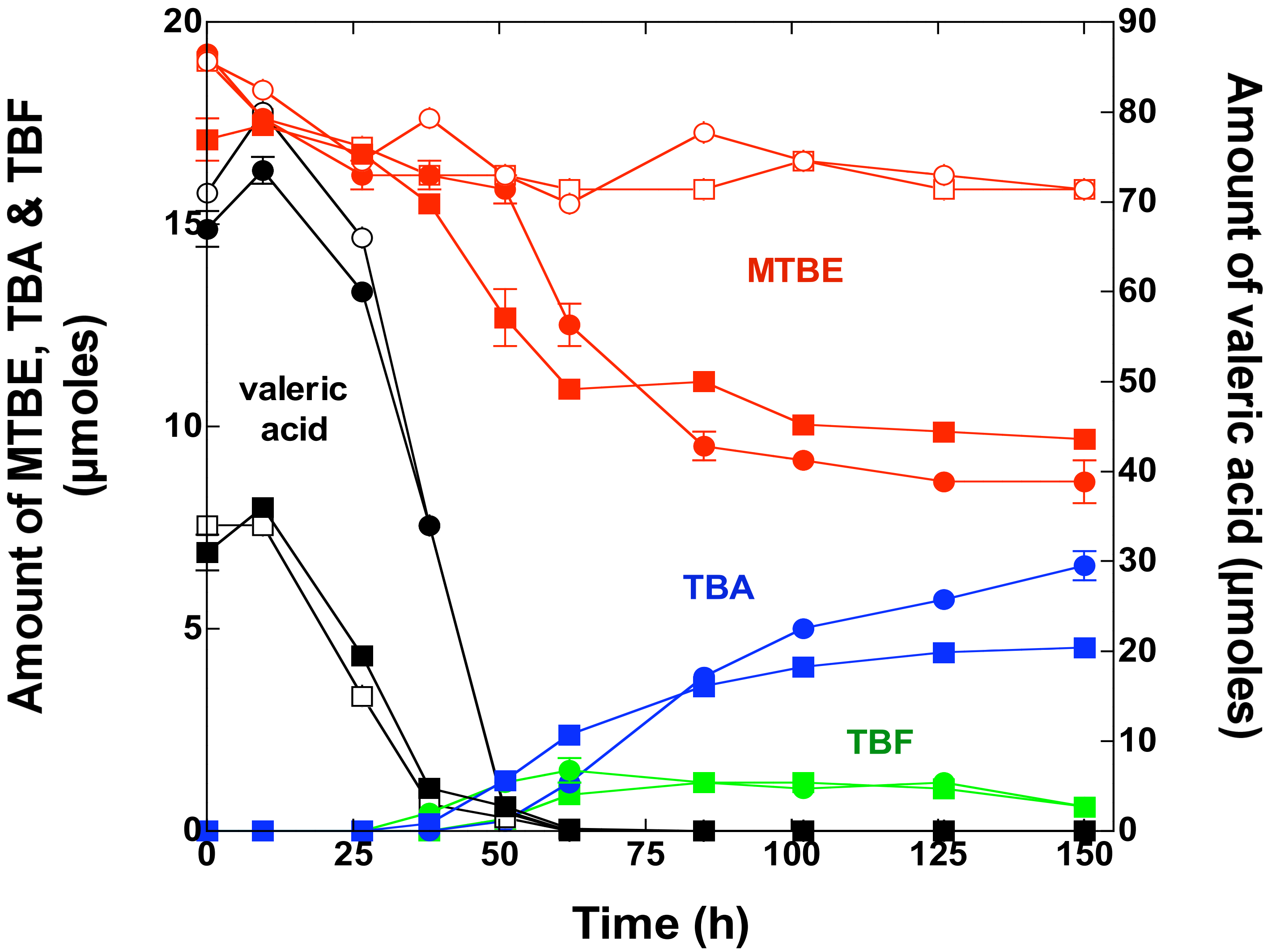
ADH

HIBA

$CH_3-C(CH_3)(COOH)-OH \rightarrow CO_2$

Carbon Dioxide

MTBE Oxidation by *M. vaccae* During Growth on Valeric Acid



N-Alkane-Oxidizing Pseudomonads

- N-alkane-oxidizing phenotype is common in soil Pseudomonads (e.g. *P. putida*, *P.aeruginosa*)

- C_5-C_8 n-alkane oxidation often (but not always) associated with alkane hydroxylase encoded by *alk* genes.

- *alk* genes found in ≤40% of culturable isolates from gasoline impacted sediments

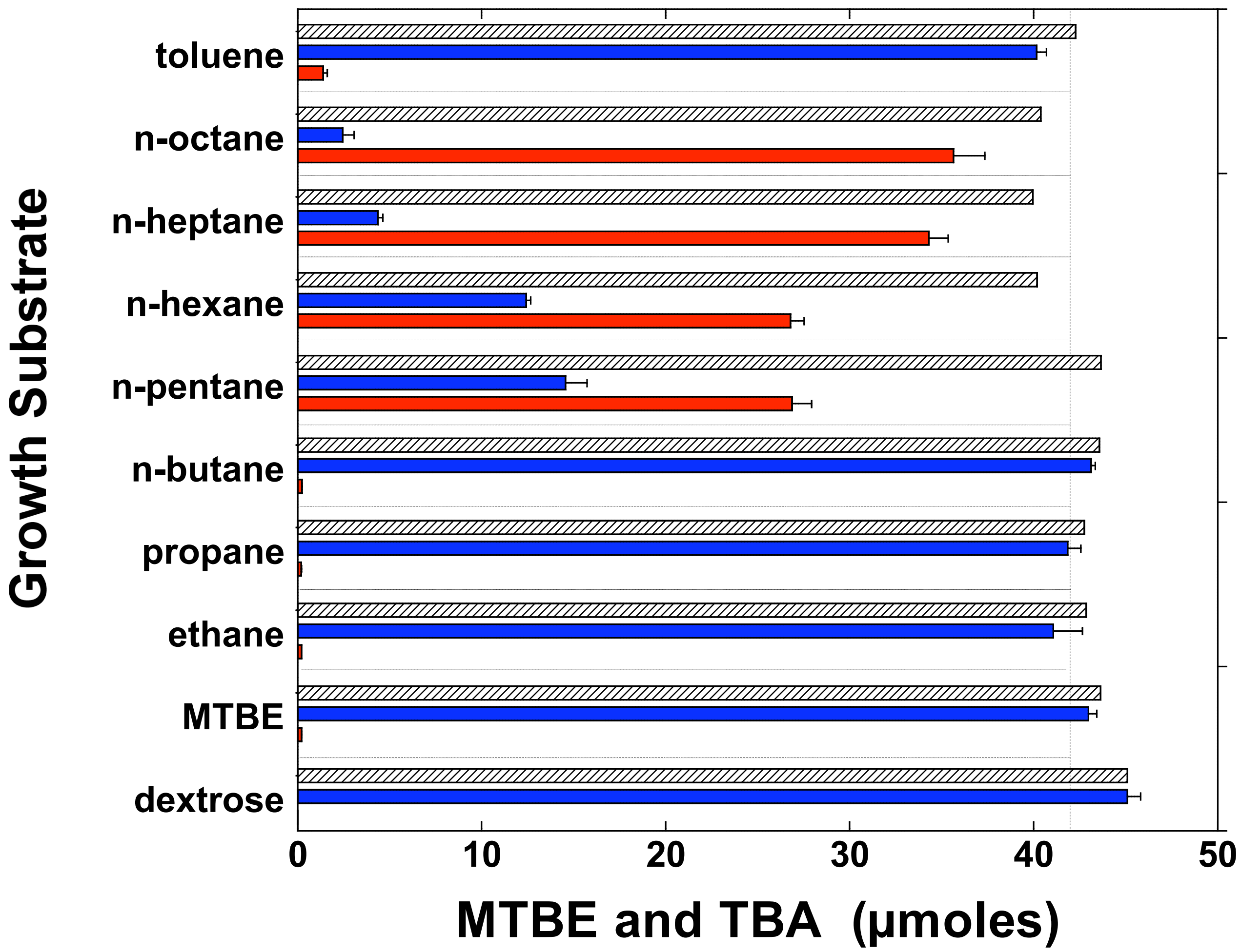
$$C_5H_{12} \xrightarrow{\boxed{\text{alkB}}} C_4H_9CH_2OH \rightarrow C_4H_9CHO \rightarrow C_4H_9COOH$$

N-pentane **1-pentanol** **valeraldehyde** **valerate**

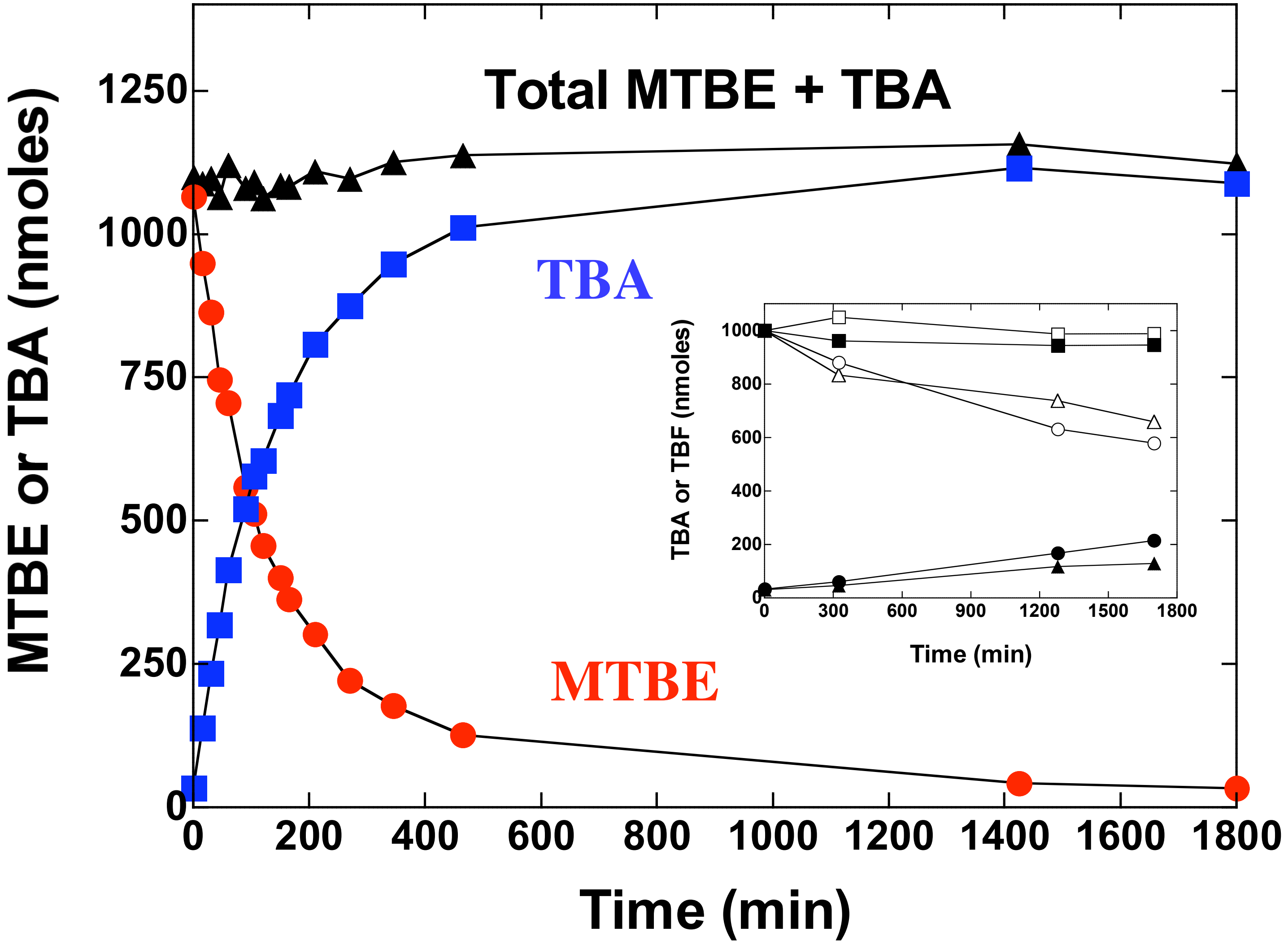
Growth Substrate Range of *Pseudomonas mendocina* KR1

Potential Growth Substrate	Absorbance (600 nm) after 5 d.	Potential Growth Substrate	Absorbance (600 nm) after 5 d.
n-Alkanes:		**1º Alcohols**	
methane	0.007 (0.001)	methanol	0.008 (0.001)
ethane	0.007 (0.001)	ethanol	0.440 (0.008)
propane	0.028 (0.010)	1-propanol	0.538 (0.009)
n-butane	0.045 (0.003)	1-butanol	0.661 (0.014)
n-pentane	0.782 (0.014)	1-pentanol	0.586 (0.021)
n-hexane	0.757 (0.020)	1-hexanol	0.646 (0.021)
n-heptane	0.737 (0.009)	1-heptanol	0.529 (0.011)
n-octane	0.781 (0.002)	1-octanol	0.561 (0.024)
Branched alkanes:		2-methyl-1-propanol	0.636 (0.016)
isobutane	0.013 (0.002)	**2º Alcohols:**	
isopentane	0.013 (0.003)	2-propanol	0.022 (0.005)
2-methylpentane	0.014 (0.001)	2-butanol	0.023 (0.001)
Aromatics:		2-pentanol	0.022 (0.001)
benzene	0.012 (0.001)	2-hexanol	0.019 (0.001)
toluene	0.429 (0.005)	2-heptanol	0.015 (0.002)
ethylbenzene	0.005 (0.001)	2-octanol	0.007 (0.005)
o-xylene	0.014 (0.008)	**Oxygenates & Ethers**	
m-xylene	0.003 (0.001)	MTBE	0.006 (0.001)
p-xylene	0.008 (0.001)	TBA	0.018 (0.002)

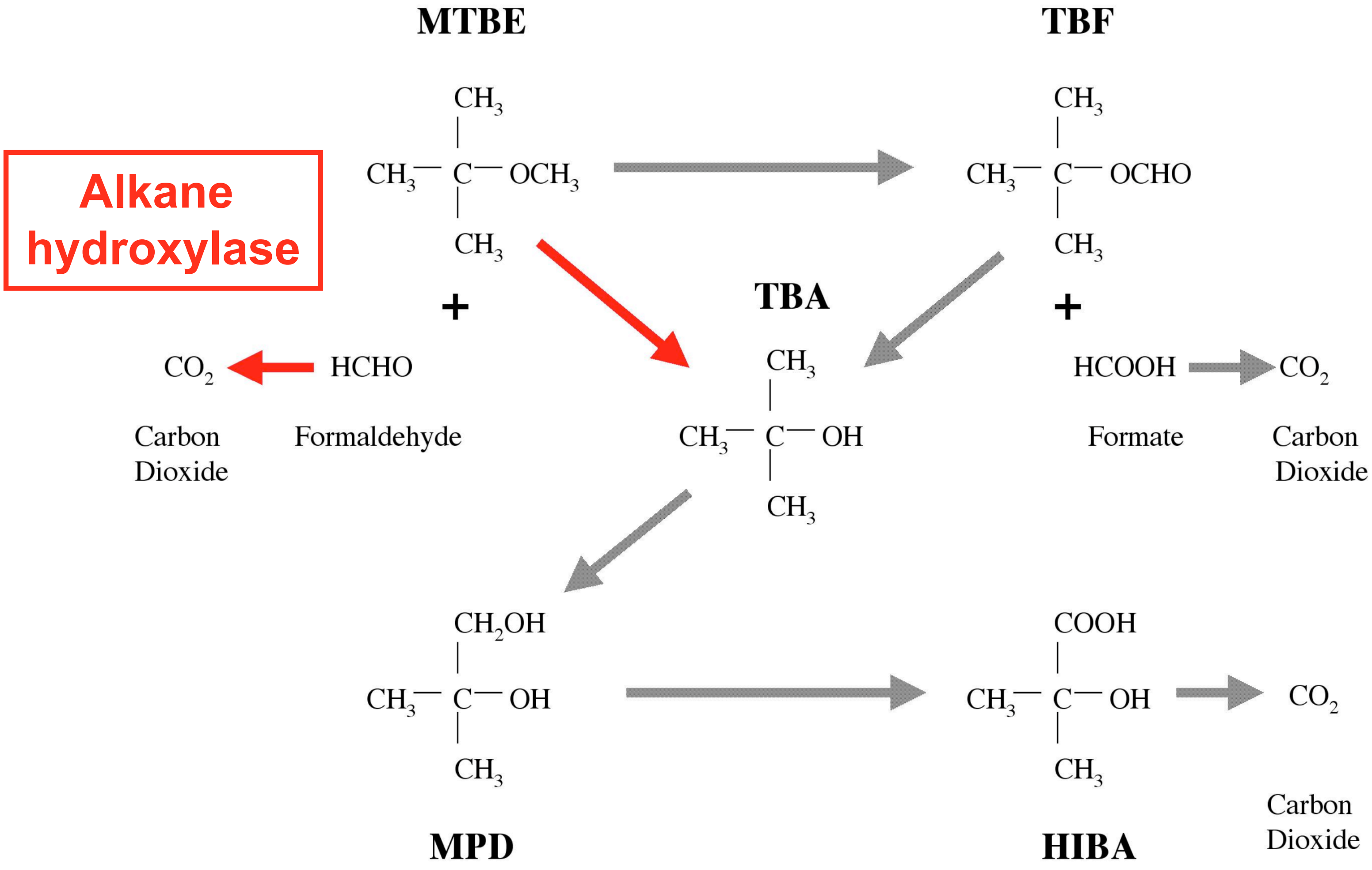
MTBE Degradation by *Pseudomonas mendocina* KR1 (at 1:1 v/v substrate:MTBE ratio)



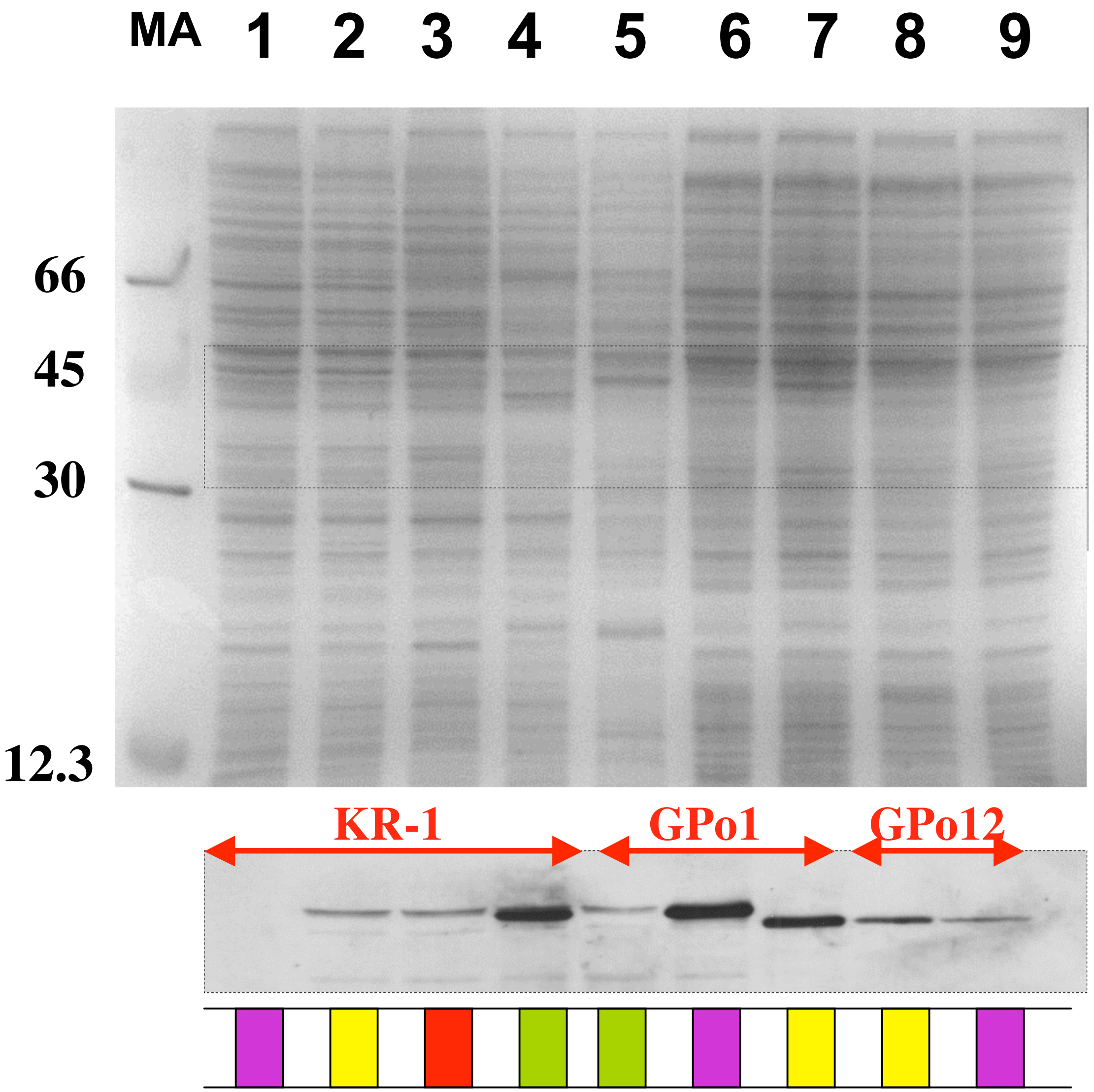
MTBE Oxidation by n-Pentane-Grown *Pseudomonas mendocina* KR1



P. mendocina Pathway and Enzyme



Alkane hydroxylase



🟩 **octane**

🟥 **octanol**

🟪 **dextrose**

🟨 **Dextrose + DCPK**

alkB Amino Acid Sequences

PCR-amplified product from *P. mendocina* KR-1 is identical to *P. putida* GPo1 alkane hydroxylase

- GPo1 GYGHFFIEHNKGHHRDVATPMDPATSRMGESIYKFSIREIPGAFIRAWGLEEQRLSRRGQ 219
- pPK8_ GYGHFFIEHNKGHHRDVATPMDPATSRMGESIYKFSIREIPGAFIRAWGLEEQRLSRRGQ 237
- KR1_ GYGHFFIEHNKGHHRDVATPMDPATSRMGESIYKFSIREIPGAFIRAWGLEEQRLSRRGQ 74
- KSLA_ GYGHFFIEHNKGHHRDVATPMDPATSRMGESIYKFSIREIPGAFIRAWGLEEQRLSRRGQ 79
- 17423_ GYGHFFIEHNKGHHRDVATPMDPATSRMGESIYKFSIREIPGAFIRAWGLEEQRLSRRGQ 78
- P1_ GYGHFFIEHNKGHHRDVATPMDPATSRMGENIYKFSTREIPGAFRRAWGLEEQRLSRRGQ 219
- alkB1_ CYAGFKVEHVRGHHVHVSTPEDASSARFGQSVYQFLPHAYKYNFLNAWRLEAVRLRKKGL 169
- PaO1 CYAGFKVEHVRGHHVHVSTPEDASSSRYGQSLYSFLPHAYKHNFLNAWRLEAERLKRKGL 169

All strains tested to date have n-alkane-dependent MTBE-oxidizing activity (K_s = >20 mM)

Genes and Kinetics

Organism	**Degradation**	**Products**	**Genes**	**$K_{s(MTBE)}$ (ppm)**
Rubrivivax gelatinosa PM1	metabolism	(**TBA**) CO_2	**???**	~10
Mycobacterium vaccae JOB5	slow metabolism	(**TBF & TBA**) CO_2?	**???**	~100
Pseudomonas putida GPo1 *Pseudomonas mendocina* KR-1	cometabolism (n-alkanes)	**TBA**	*alk*B	~1000
Pseudonocardia K1	cometabolism (THF)	**TBA**	*thm*	~2
Pseudomonas putida	cometabolism (camphor)	**TBA**	*cam*	~50
Nitrosomonas europaea	cometabolism (NH_4^+)	**TBA**	*amo*	~5,000

Conclusions

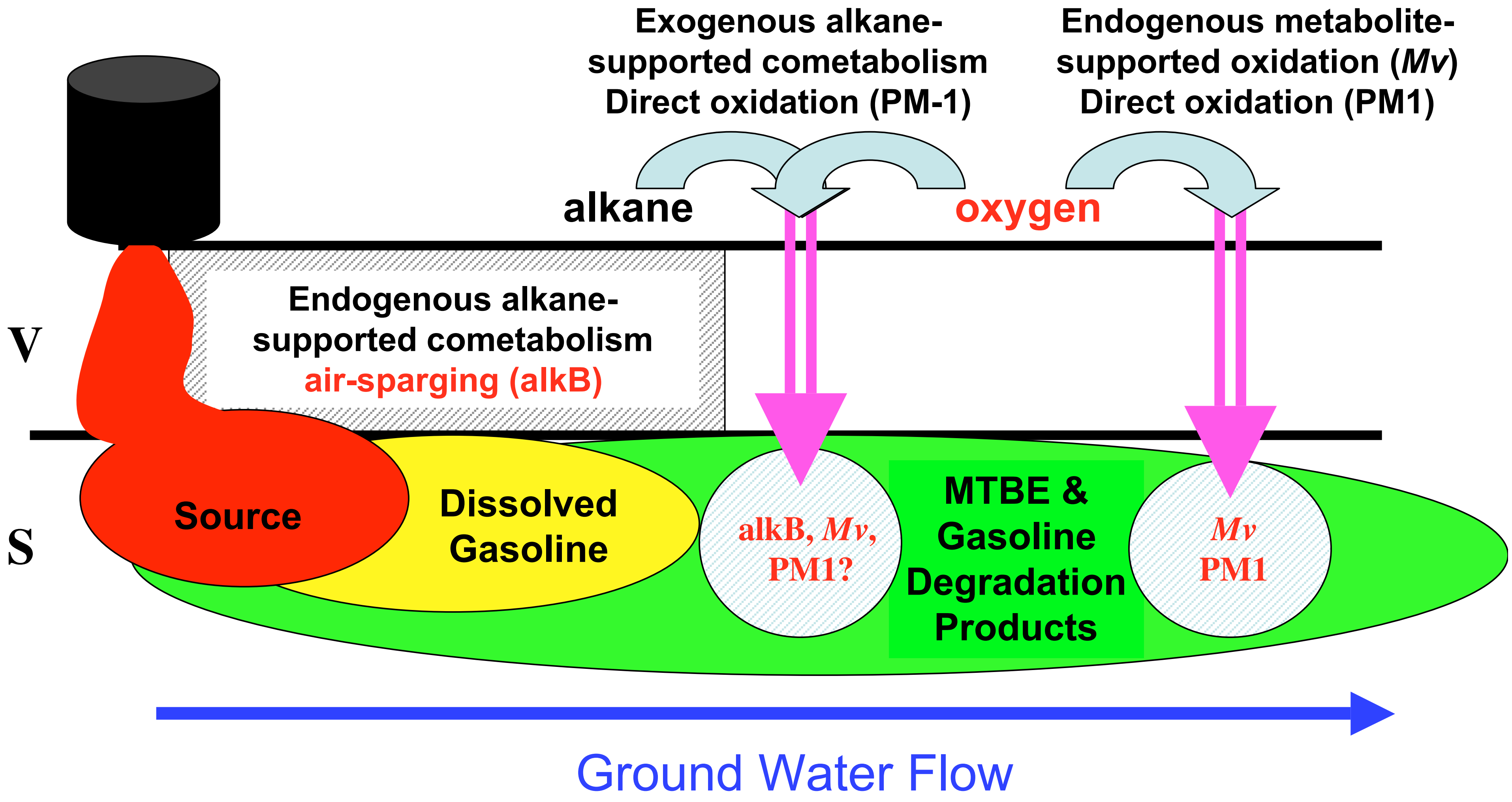
- ***Rubrivivax gelatinosa* PM1** oxidizes MTBE and TBA using different inducible enzymes. Productive further metabolism of TBA. (“low” K_s for both MTBE and TBA). What about H_2-oxidizing activity?

- ***Mycobacterium vaccae* JOB5** oxidizes MTBE and TBA using the same inducible enzyme. Growth limited by inefficient/incomplete further oxidation of TBA? (“medium” K_s for both MTBE and TBA)

- ***Pseudomonas mendocina* KR-1** (and many others) are true MTBE cometabolizers and transform MTBE to TBA. No further oxidation of TBA in pure culture but MTBE mineralization can occur with TBA-degrading partner. (“very low” to “very high” K_s for MTBE alone)

- **All of these organisms grow on select BTEX components.** Does this capability impact their distribution and activity in gasoline-impacted environments?

Aerobic Biodegradation of MTBE in a Gasoline Plume?



Exogenous alkane-supported cometabolism Direct oxidation (PM-1)

Endogenous metabolite-supported oxidation (*Mv*) Direct oxidation (PM1)

alkane

oxygen

Endogenous alkane-supported cometabolism
air-sparging (alkB)

Ground Water Flow