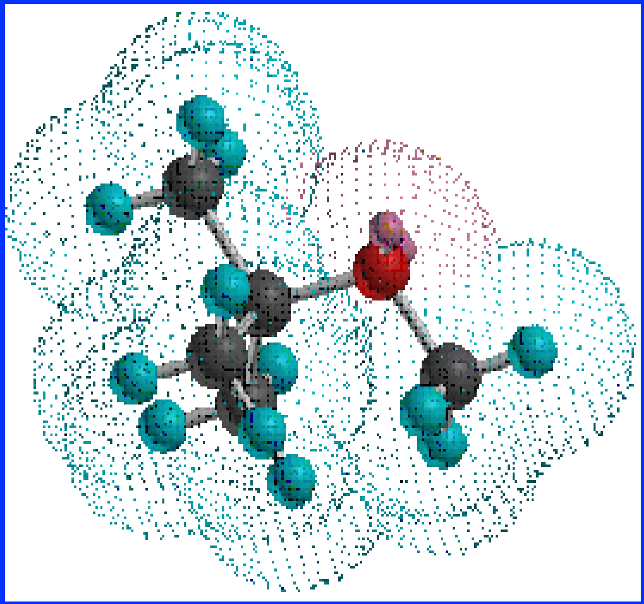
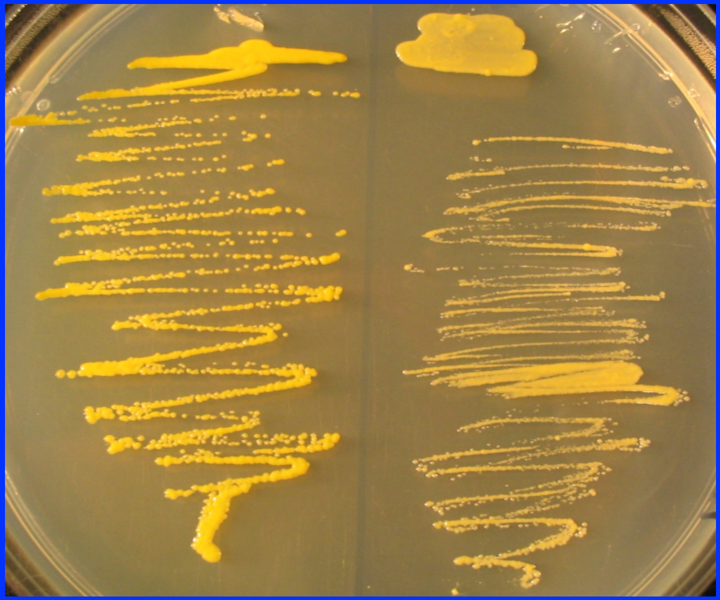
Methyl *tert*-Butyl Ether (MTBE) biodegradation.



Methyl *tert*-butyl ether

(MTBE)

Mycobacterium austroafricanum
MTBE-degrading strains



IFP 2012 / IFP 2015

Main fuel oxygenates = ethers & alcohols

CH_3
|
$CH_3 - C - O - CH_3$
|
CH_3

Methyl *tert*-butyl ether
(MTBE)

CH_3
|
$CH_3 - C - O - CH_3$
|
CH_3

Ethyl *tert*-butyl ether
(ETBE)

CH_3
|
$CH_3 - C - O - CH_3$
|
$CH_2 - CH_3$

tert-Amyl methyl ether
(TAME)

CH_3
|
$CH_3 - C - O - H$
|
CH_2

tert-Butyl alcohol
(TBA)

CH_3
|
$CH_3 - C - O - H$
|
$CH_2 - CH_3$

tert-Amyl alcohol
(TAA)

$CH_3 - CH_2 - OH$

Ethanol

Properties & use of fuel oxygenates

- Addition in gasoline
  - Octane index enhancer.
  - Air quality improvement: decrease of CO & unburnt hydrocarbons.
  - Reducing the lead concentration in human blood by 50%.

- Contamination of aquifers
  - High water solubility (MTBE, 40 $g.L^{-1}$)
  - Low adsorption on organic matter
  - Recalcitrance to biodegradation (limited natural attenuation)

- Presence of an ether bond thermodynamically highly stable : $\boxed{\text{-C-O-C-}}$

- Presence of a *tert*-butyl group with a high steric hindrance :

$$\boxed{\begin{array}{ccc} & & CH_3 \\ & & | \\ CH_3 & - & C - \\ & & | \\ & & CH_3 \end{array}}$$

Ecology of MTBE biodegradation

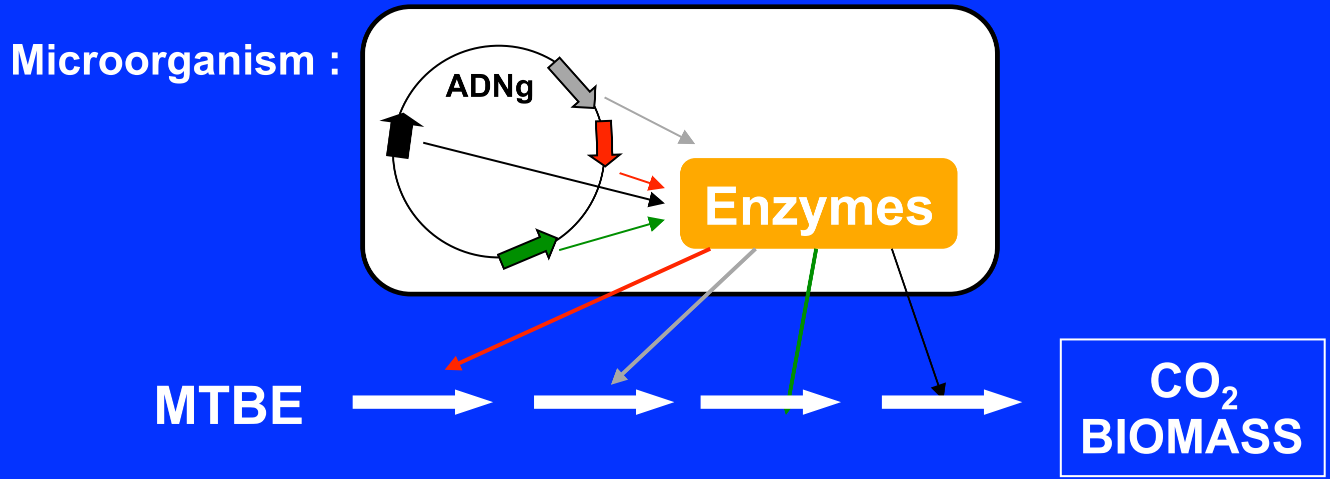
i) Isolation of strains with degradation capacities.

ii) Characterization of the enzymes involved in the degradation pathway.

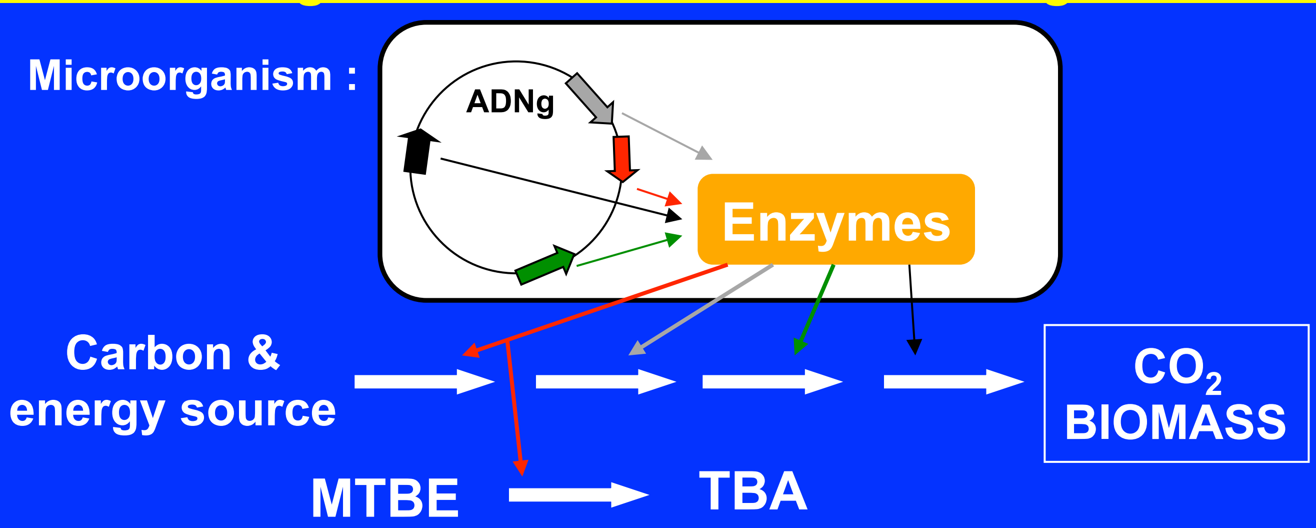
iii) Cloning & sequencing of the corresponding genes.

MTBE biodegradation

I) Metabolism : growth & mineralization



II) Cometabolism : no growth & accumulation of degradation intermediates



Biodegradation of MTBE (1) : cometabolism

Microorganisms

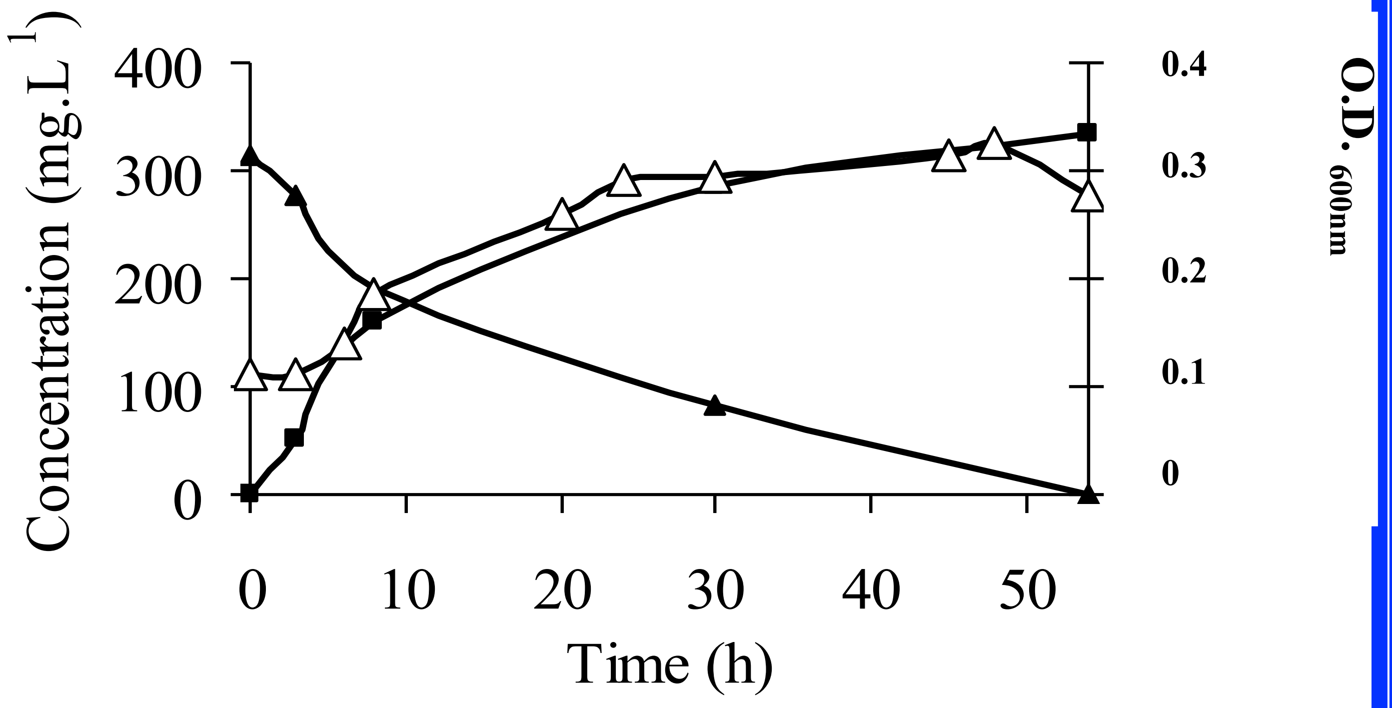
Substrate		Microorganism	Oxygenase involved
Propane		*Mycobacterium vaccae* JOB5	Propane monooxygenase ?
		Xanthobacter sp.	Propane monooxygenase ?
		Nocardia ENV425	Cytochrome P450 ?
Butane		*Graphium* sp.	Cytochrome P450 ?
		Arthrobacter ATCC27778	Butane monooxygenase ?
ETBE	→	*Rhodococcus ruber* IFP 2001	<u>Cytochrome P450 (*eth* genes)</u>
Octane	→	*Pseudomonas putida* GPo1	<u>Alkane hydroxylase (*alkB*)</u>

Cometabolic biodegradation of MTBE

1-Cyrochrome P450 of *R. ruber* IFP 2001
(*eth* genes)

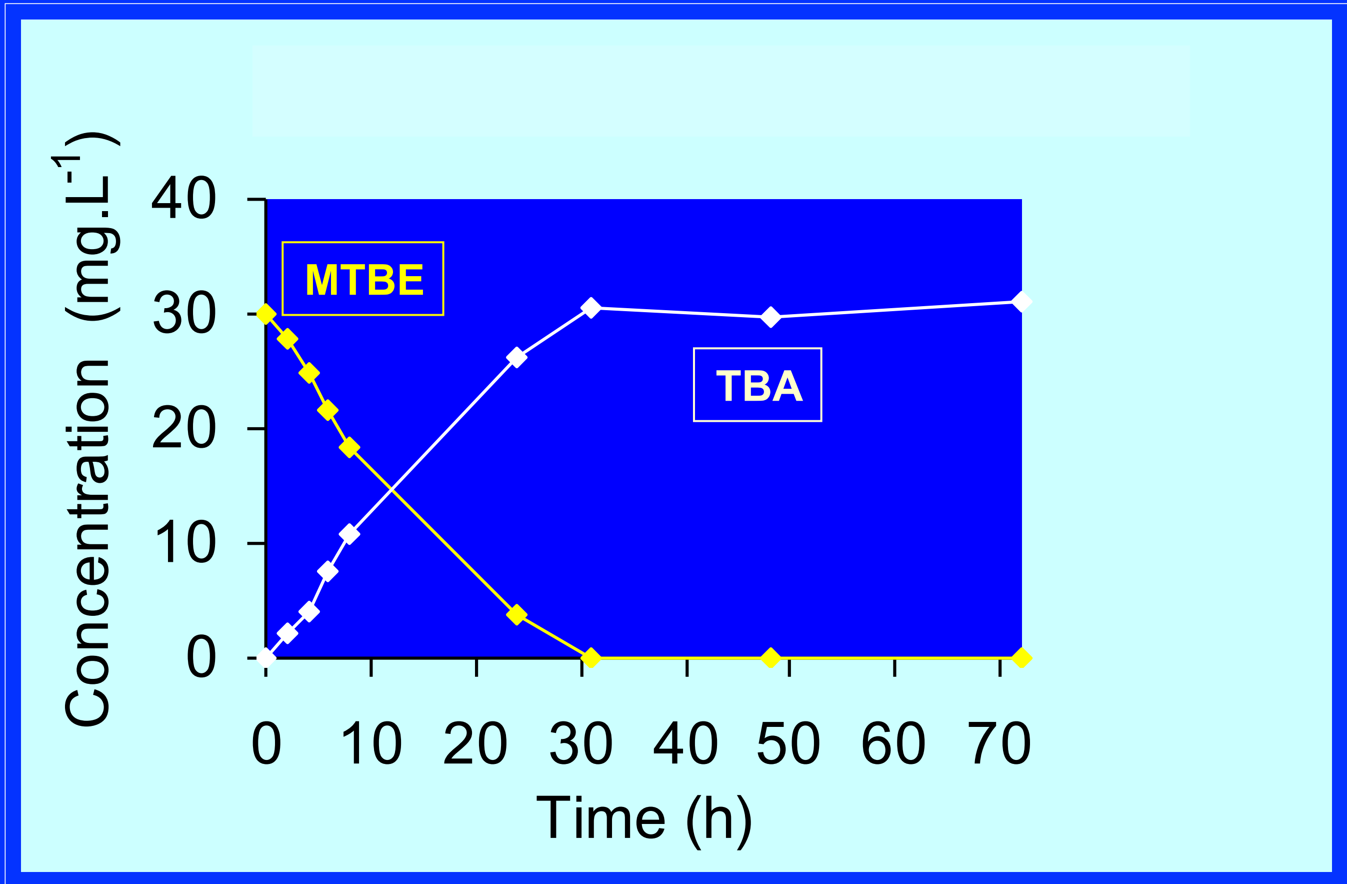
Hernandez-Perez *et al.* 2001
Chauvaux *et al.* 2001
Béguin *et al.* 2003

Growth of *R. ruber* IFP 2001 on ETBE



Conversion of MTBE by *R. ruber* IFP 2001

Resting cells experiments

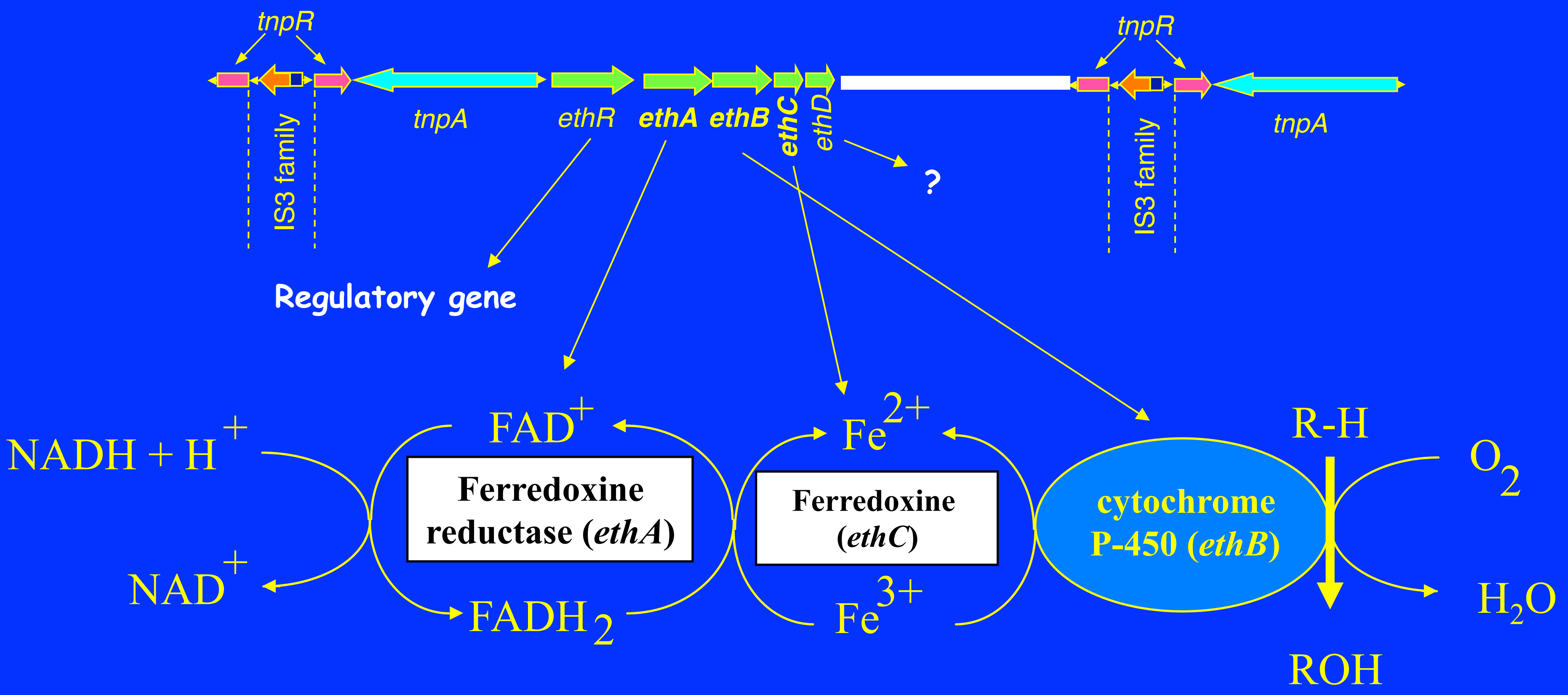


➨ *R. ruber* IFP 2001 can convert MTBE to TBA by cometabolism

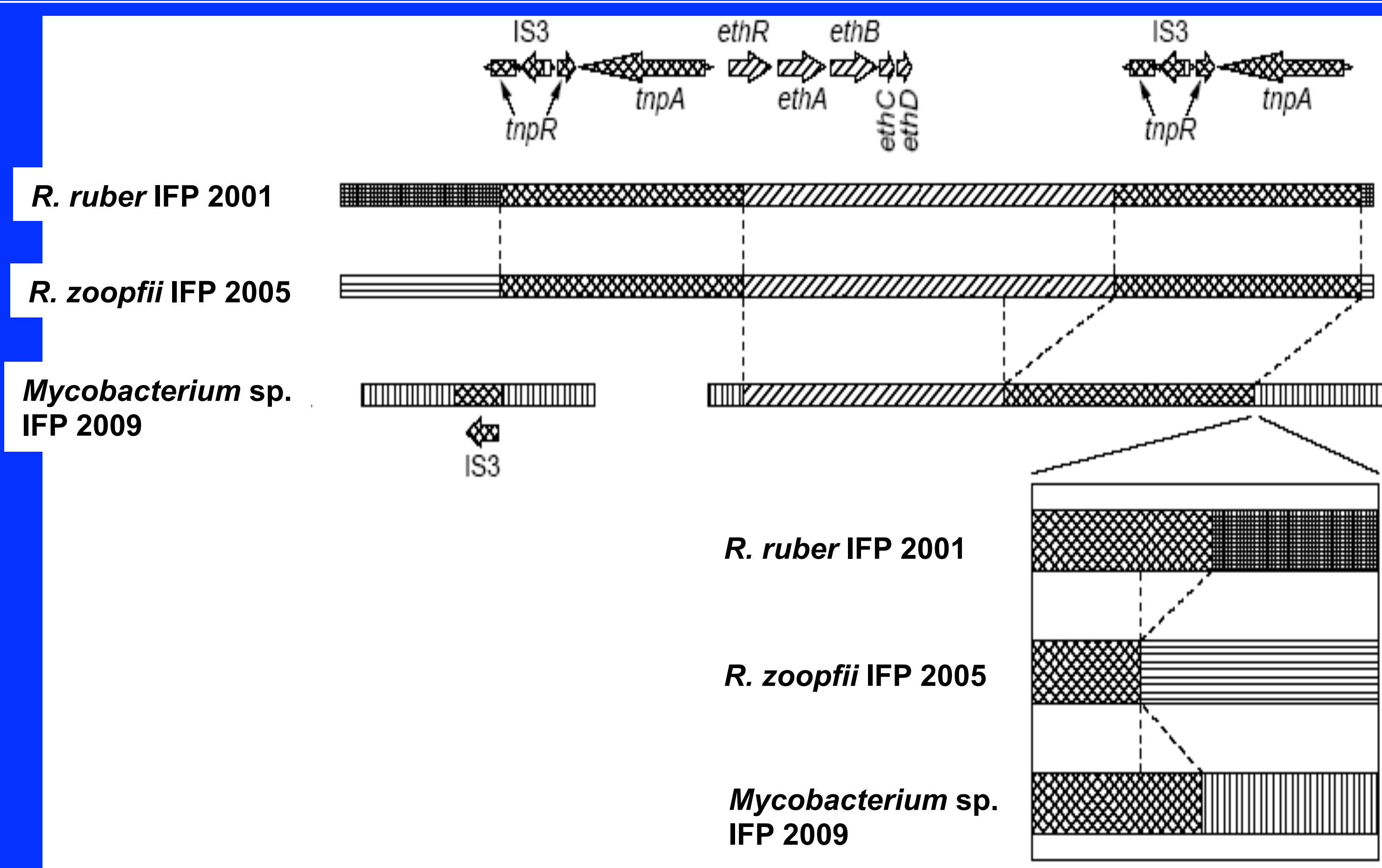
eth genes of R. ruber IFP 2001

→ **First ETBE / MTBE-degrading system characterized**

- Induction of a cytochrome P450 during growth on ETBE
- *eth* cluster (from Chauvaux *et al.*, 2001)



Ecology of *eth* genes



-*R. zoopfii* IFP 2005 and *R. ruber* IFP 2001 : same activated sludge

- *Mycobacterium* sp. IFP 2009 : another activated sludge

⟶ Possibility of **gene transfer**.

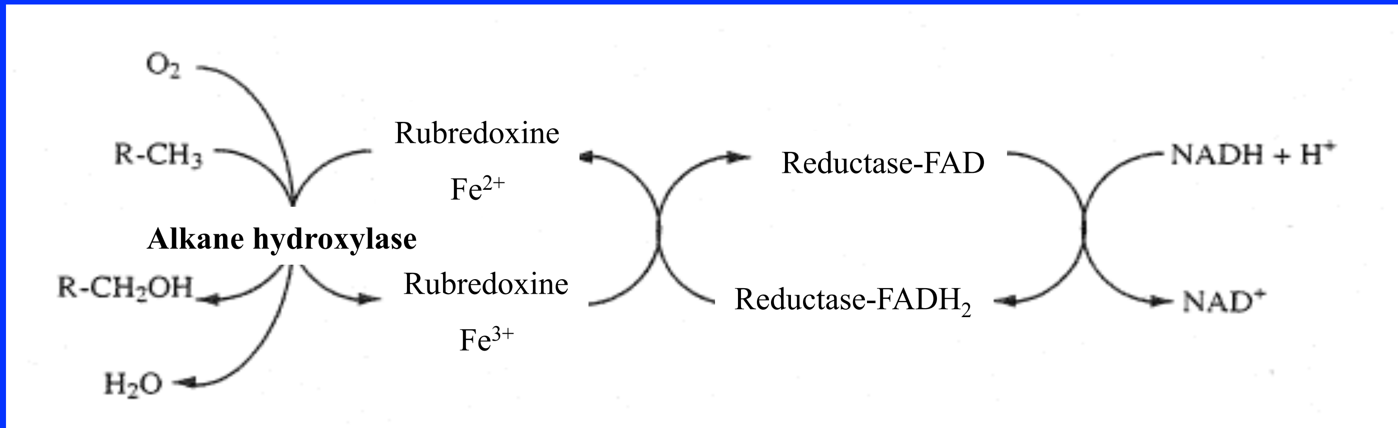
Cometabolic biodegradation of MTBE

2-Alkane hydroxylase of *P. putida* GPo1
(*alkB* gene)

Smith and Hyman, 2004

alk genes of *P. putida* GPo1

-Alkane hydroxylase = membrane-bound oxygenase encoded by *alkB gene* .



-*alk* genes located on the plasmid OCT in *P. putida* GPo1.

-Smith & Hyman [2004] : *AlkB* able to oxidize MTBE but not TBA.

- Loss of OCT → loss of the MTBE degradation capacity.

-Affinity of the alkane hydroxylase for MTBE very low (20 mM<Km).

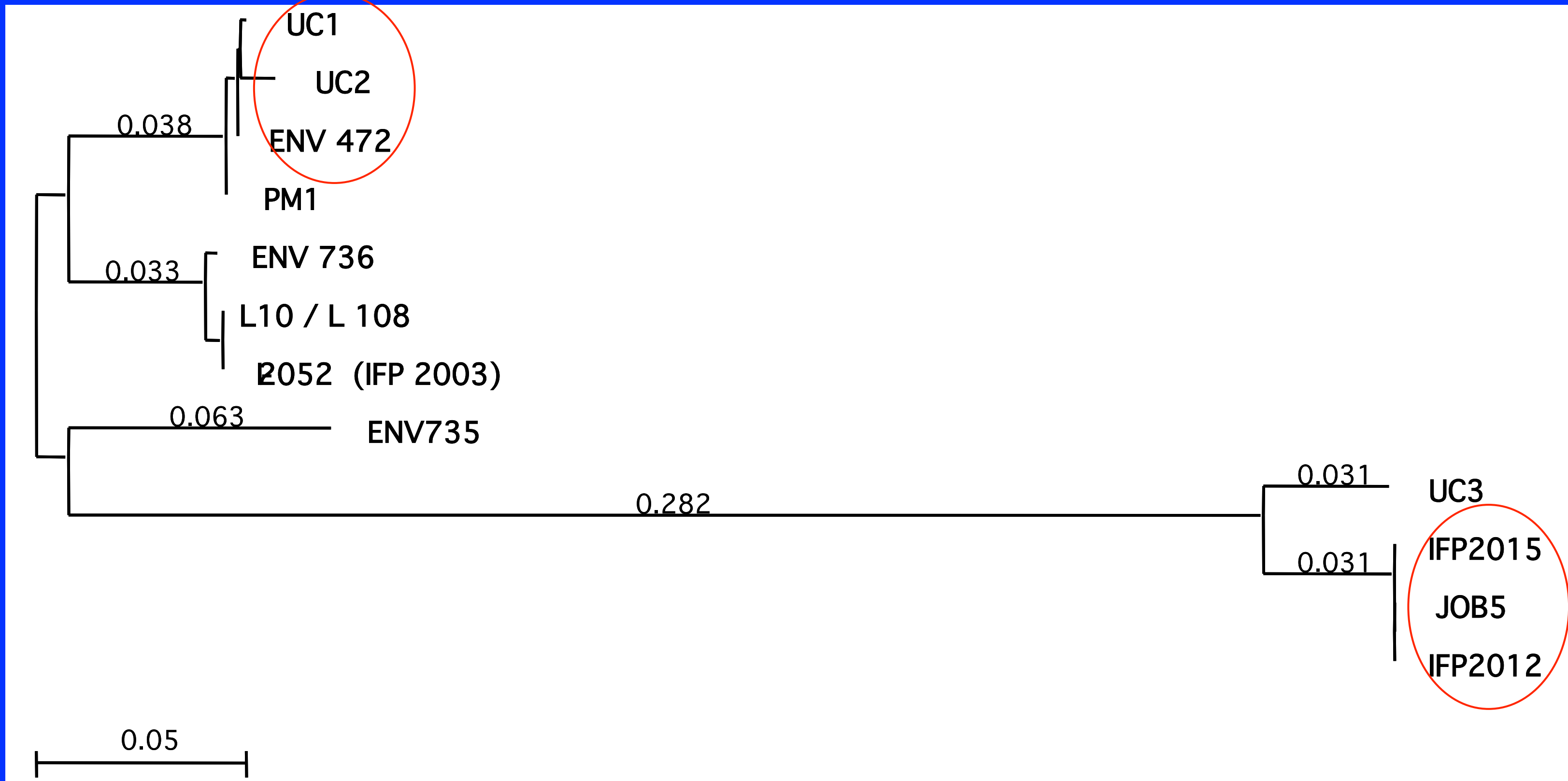
⟶ *alkB* : commonly detected in the environment.

Why is MTBE degradation rarely found? Affinity?

Biodegradation of MTBE (2) : growth substrate

Microorganisms able to growth on MTBE

Phylogenetic tree of environnemental strains able to growth on MTBE (& TBA)



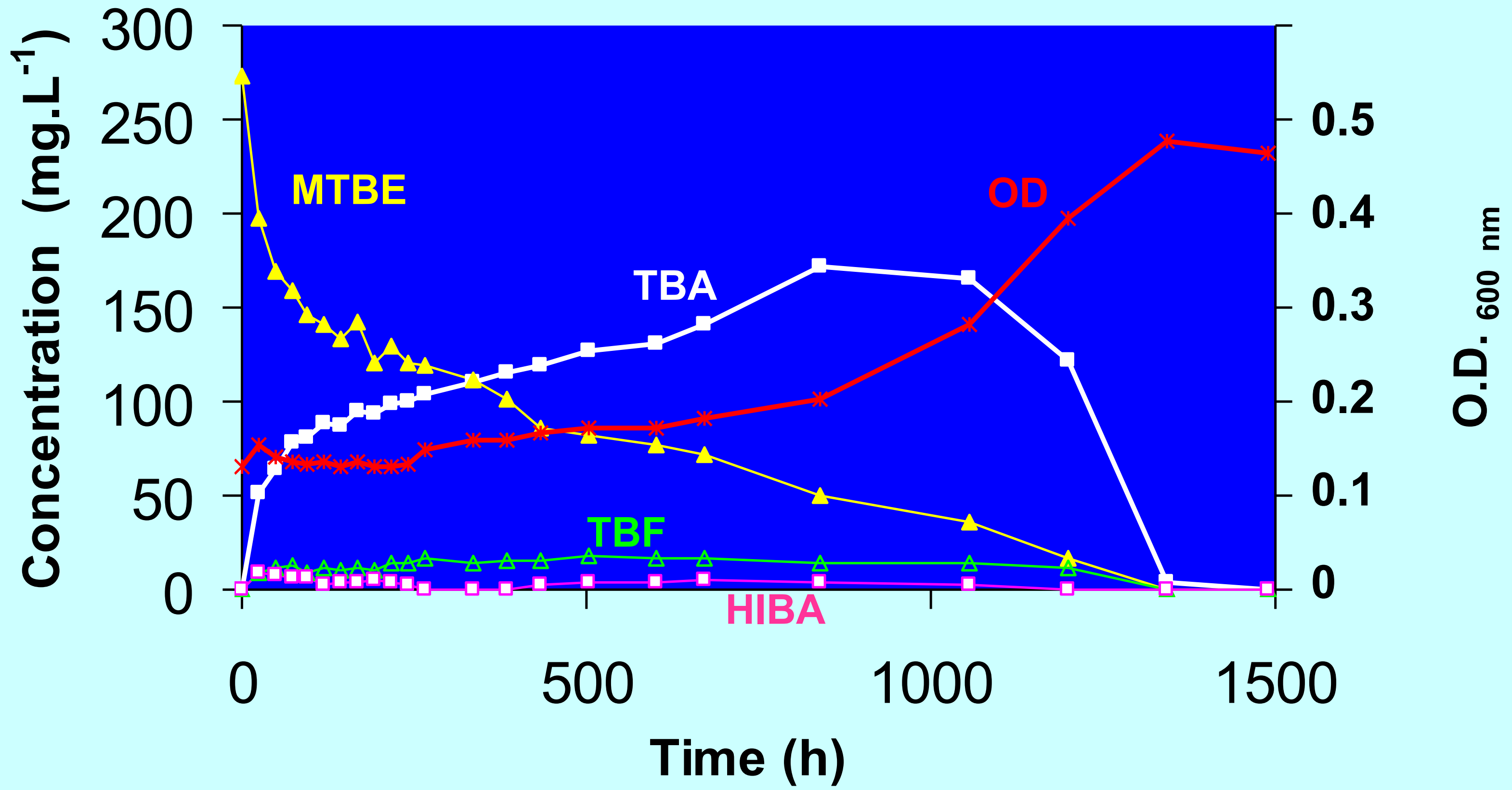
Growth on MTBE

Mycobacterium austroafricanum

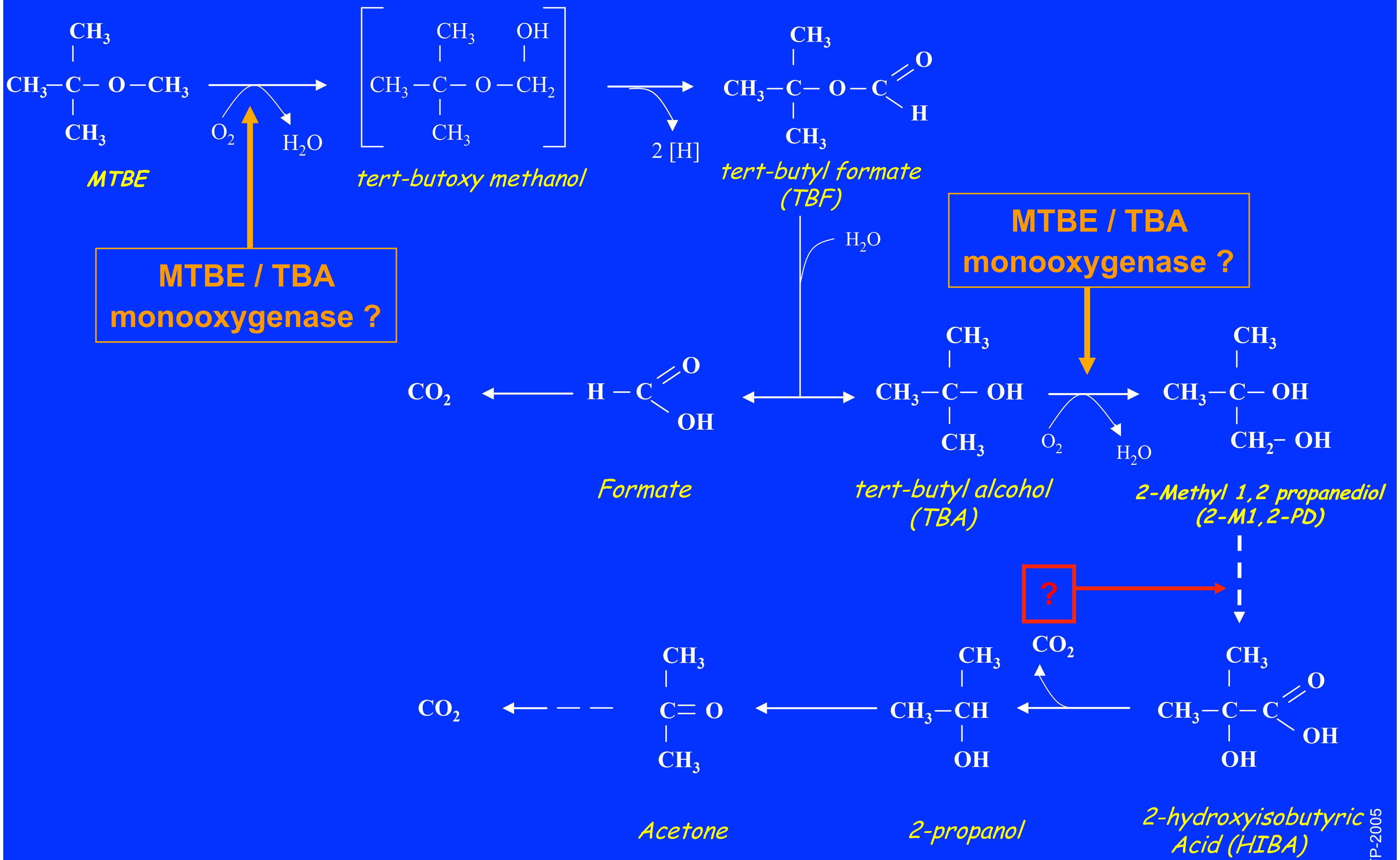
IFP 2012

M. austroafricanum IFP 2012

Growth on MTBE



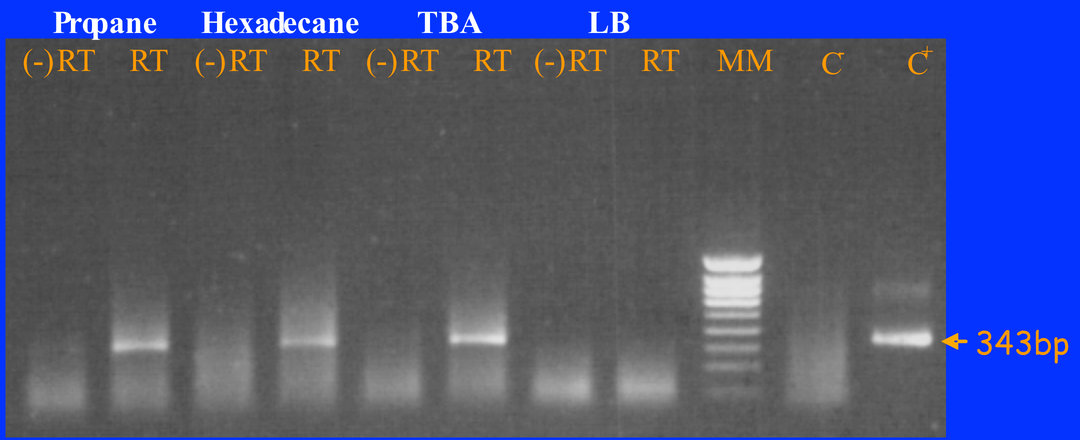
MTBE metabolic pathway of *M. austroafricanum* IFP 2012



Genes involved in MTBE degradation pathway by *M. austroafricanum* IFP 2012

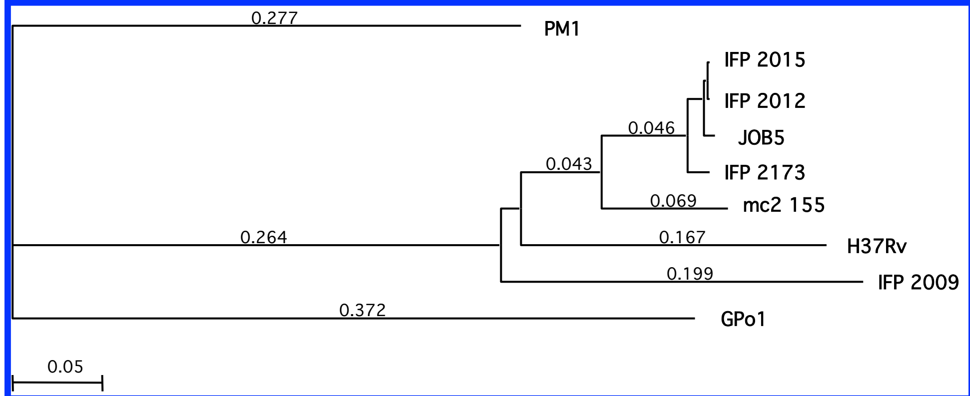
- 343 bp-fragment amplified by PCR from the DNA of *M. austroafricanum* IFP 2012 using a primer pair specific for *alkB*

- RT-PCR experiments after growth on n-alkanes from C_2 to C_{16}

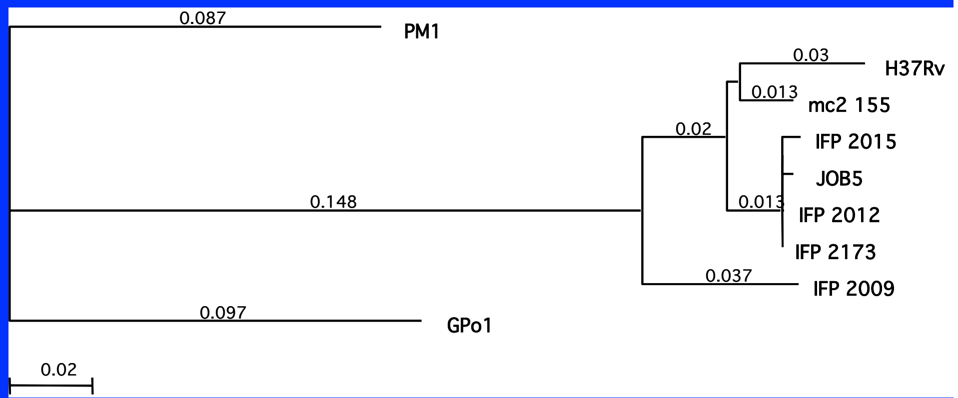


alkB genes in the species *M. austroafricanum*

- PCR amplification of *alkB* gene using specific primers.
- Phylogenic comparison of *alkB* genes of Mycobacteria.



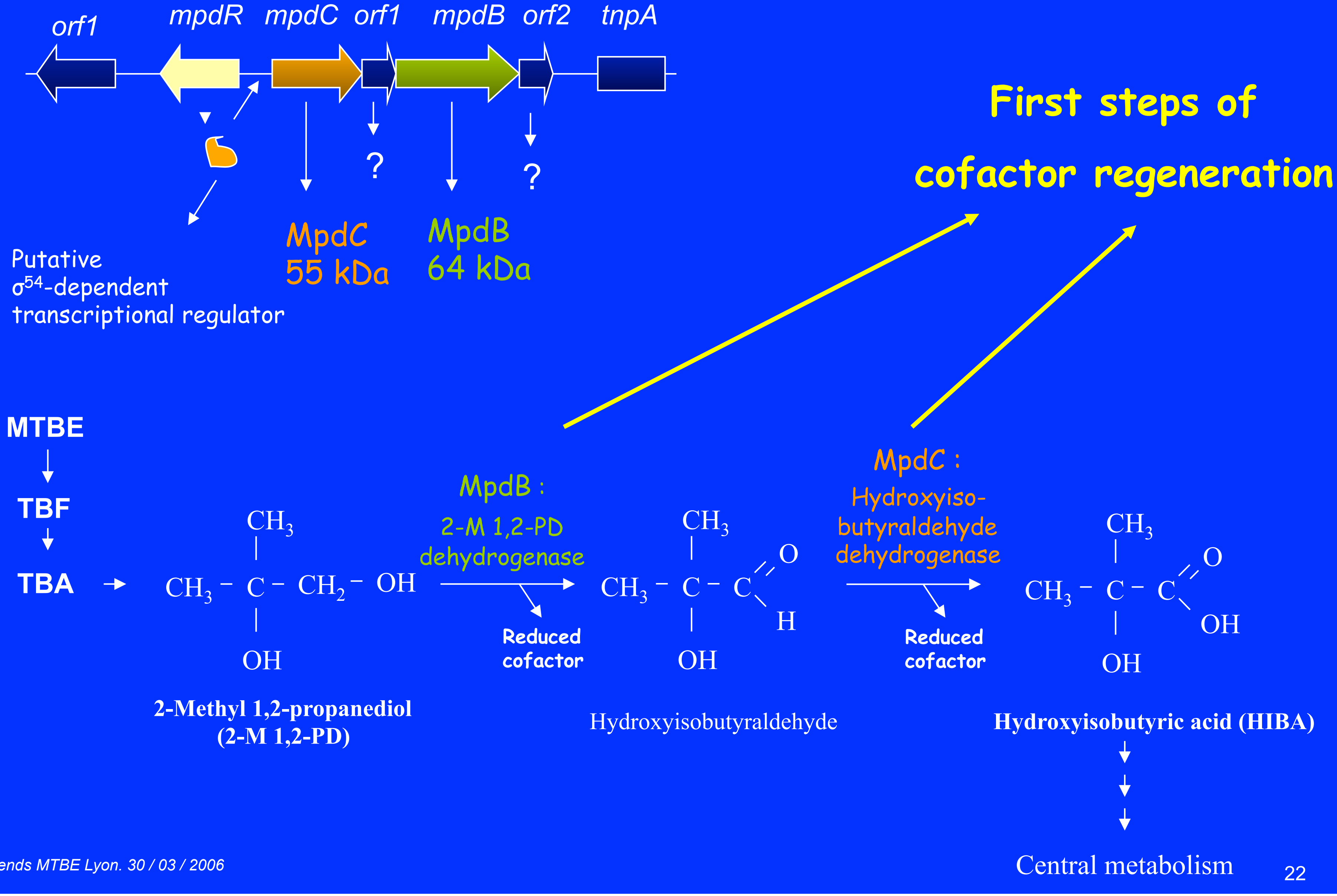
Partial alkB gene sequences



16S rDNAs

➡ Information on the ecology of MTBE and TBA degradation vs. alkane assimilation.

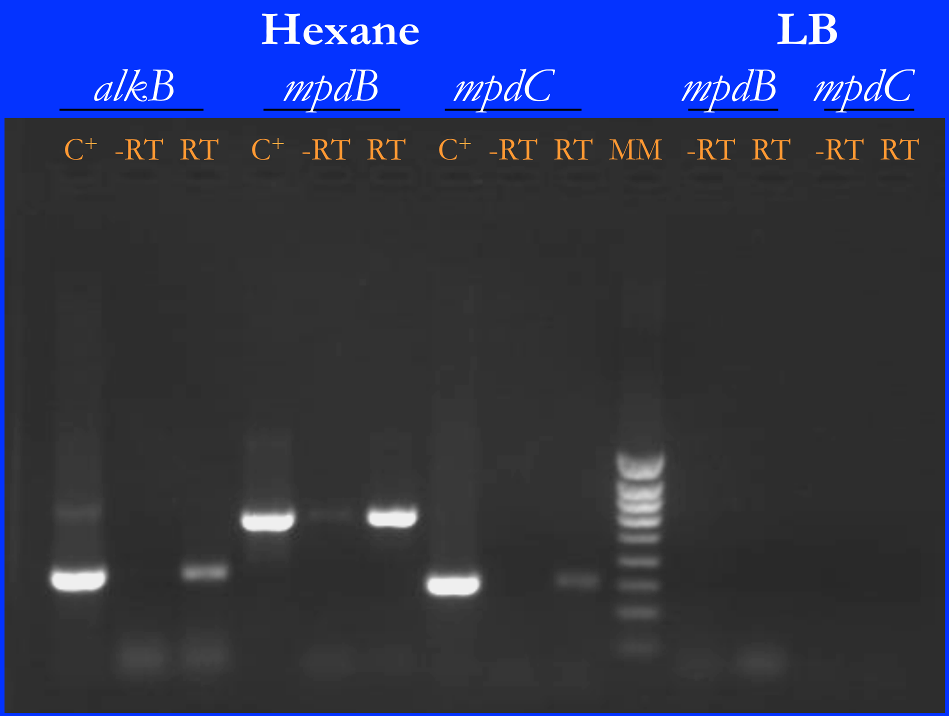
mpd genes involved in the MTBE degradation pathway of *M. austroafricanum* IFP 2012



Growth on MTBE

- **Expression of *alkB*, *mpdB* & *mpdC* on hexane.**

↳ **Implication of MpdB & MpdC in alkane assimilation ?**



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Diane Labbé, Charles Greer.

Octane index: use of tetraethyl lead

TETRAETHYL LEAD : Pb $(C_2H_5)_4$:
added to gasoline for 50 years

To increase the octane index of gasoline

Poisoning of post-treatment catalysts

High toxicity for animal and human : saturnism

Banishment of tetraethyl lead use from the 70s'.

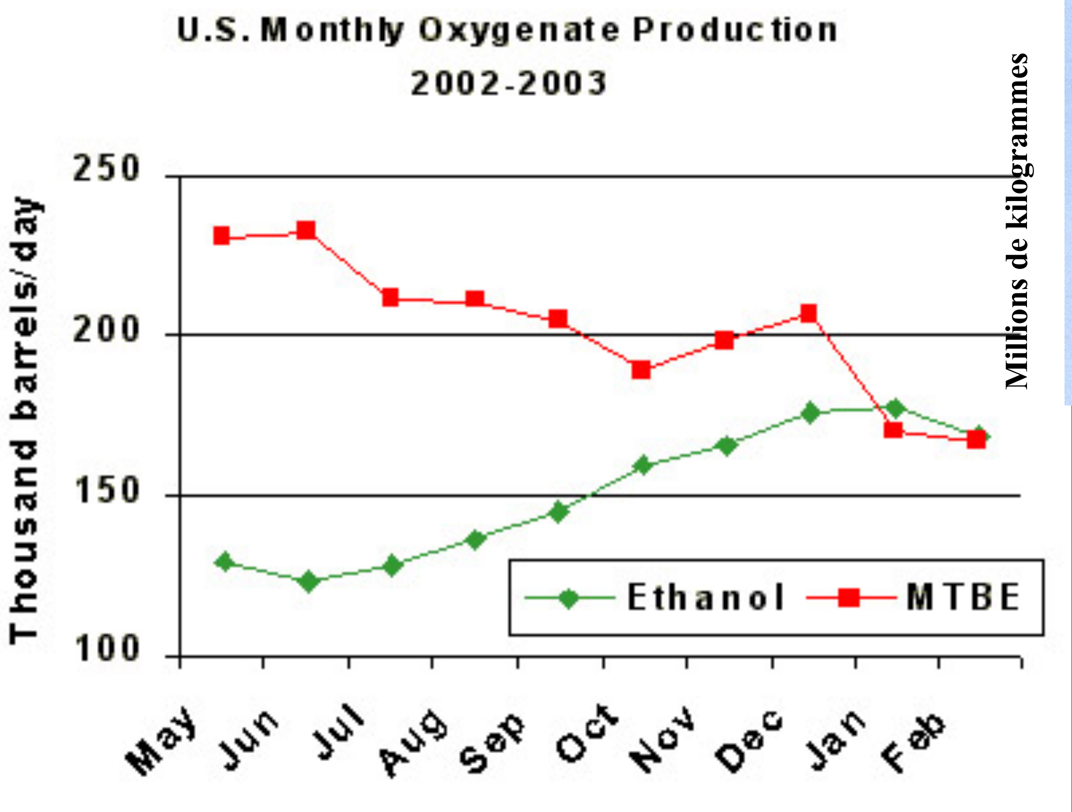
Biodegradability of ether-fuels by various microflorae

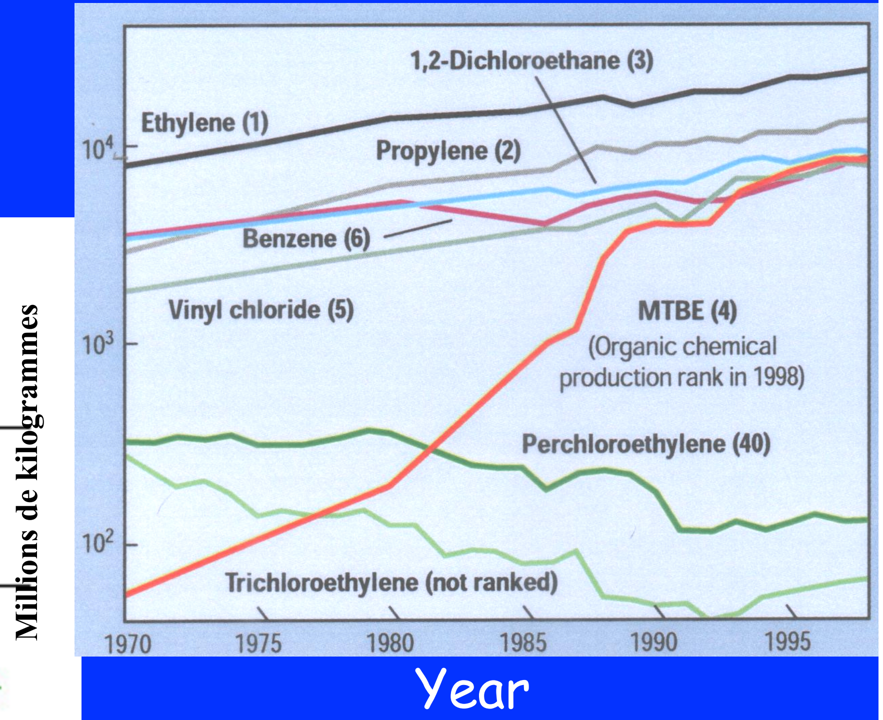
Origin of microflorae	Biodegradation of MTBE (%)	Biodegradation of ETBE (%)	Biodegradation of TBA (%)	Biodegradation of TAME (%)
Activated sludge A	40	100	100	100
Activated sludge V	31	100	100	84
Activated sludge F	0	8	100	0
Soil M	37	100	100	0
Soil B	0	5	100	5
Soil G	4	0	100	0
Soil N	0	0	100	0

Progressive phase out in USA

MTBE in 1999: production, 21 millions tons and use, 61 % in USA, 15% in Europe.

3rd chemical produced worldwide.





In 2003, 17 states in the U.S. banned the use of MTBE in fuel.

ETBE metabolic pathway by *R. ruber* IFP 2001