

The Methylootrophs

They use compounds with one or more carbon atoms but no C-C bonds as their sole source of carbon and energy: Methane, Methanol, methylamine, dimethylamine.

Those that use methane are also called **Methanotrophs** (eg. *Methylococcus*, *Methylosinus*)

Obligate methylootrophs cannot grow on multicarbon compounds (include most methanotrophs)

Facultative methylootrophs also grow on multicarbon compounds (eg. *Methylobacterium extorquens* = *Pseudomonas* AM1)

Restricted facultative methylootrophs grow on C1 or C2 compounds (eg. *Hyphomicrobium*)

Facultative autotrophs grow on carbon dioxide, plus hydrogen or methanol by way of RuBP path (eg *Paracoccus*)

There is a huge number of different types of methylootroph. Very few are typical bacteria that had been described previously.

Although their oxidative metabolism is basically similar, there are many different carbon assimilation pathways.

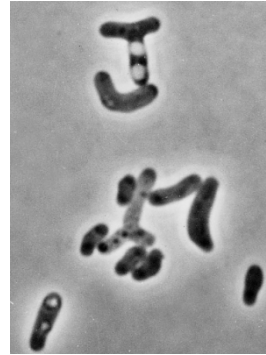
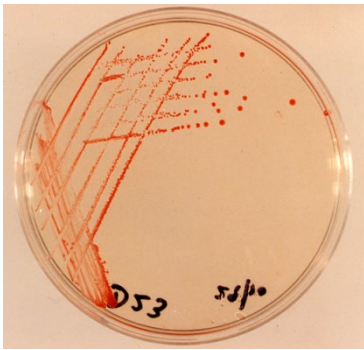
Most are Gram-negative. The few Gram positive methylootrophs (*Bacillus* sp) have different oxidation enzymes

- 1906 Sohngen. *Bacillus methanicus* growing on methane
- 1961 Peel and Quayle. *Pseudomonas* AM1 (cited only 6 papers) A pink facultative methylootroph
- 1964 Anthony and Zatman. Description of Methanol dehydrogenase in *Pseudomonas* M27 (=AM1)
- 1964 Hirsch and Conti. *Hyphomicrobium*. A stalked facultative methylootroph
- 1970 Whittenbury (with Wilkinson). 100 new methanotrophs (2 Types)
- 1971 Ribbons Early work on methane oxidation
- 1970- Ogata, Sahm, Hazeu, van Dijken, Harder. Methylootrophic yeast.
- 1980 Dalton Definitive work on methane oxidation
- 1982 Anthony book. Cited about 1200 methylootroph references

The main 'workhorses' in the study of methylotrophs

- *Pseudomonas* AM1 (Peel & Quayle, 1961)
- *Pseudomonas* sp. M27 (Anthony & Zatman, 1964)

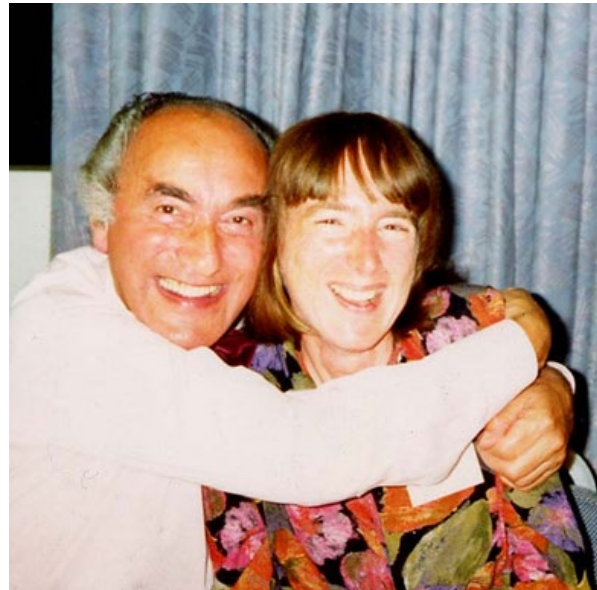
***Methylobacterium extorquens*.** A pink facultative methylotroph



Me in 1963



J Rod Quayle



My PhD supervisor and my first research student (Len Zatman and Pat Dunstan, now Goodwin)



Young Howard & Rod Quayle

Howard Dalton, Rod and me

Methylococcus capsulatum
Methylosinus trichosporium

(Type I obligate methanotroph)
(Type II obligate methanotroph)



Type I membranes

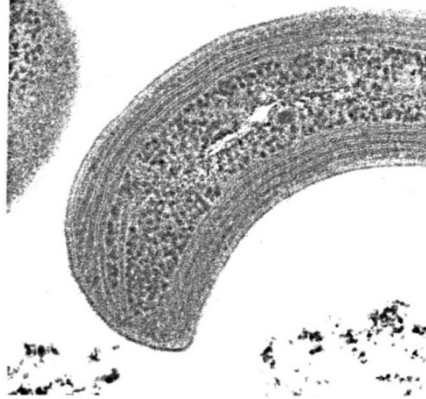
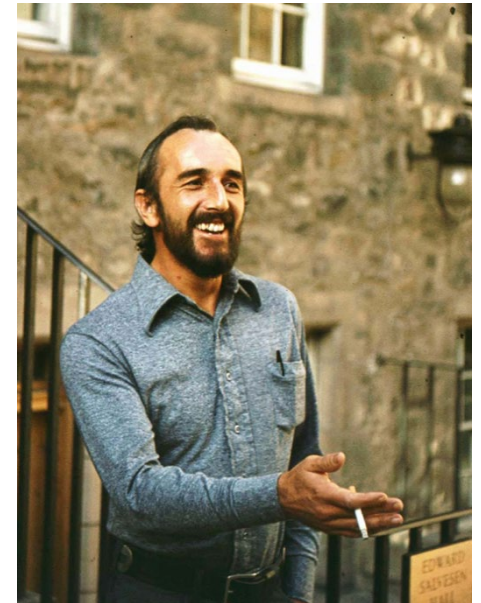


Fig. 2. *Methanomonas methano-oxidans* showing Type II membranes. This electron micrograph was kindly provided by Professor D. W. Ribbons. Type II membranes are typically arranged as a system of paired peripheral membranes.

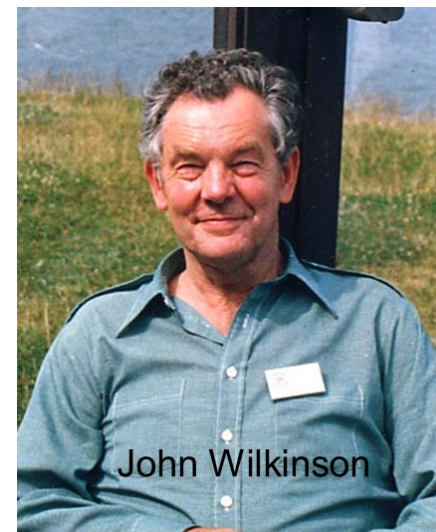
Type II membranes



Doug Ribbons 1973

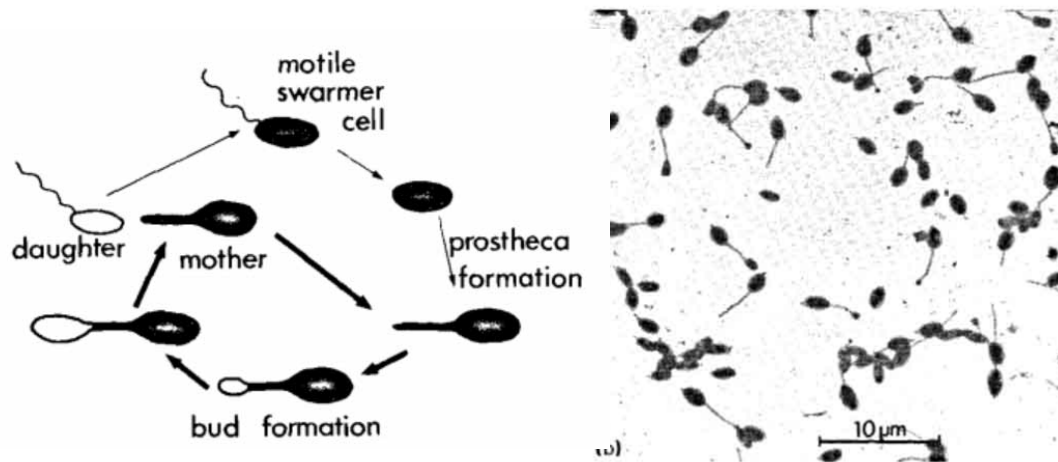


Rod Quayle & Roger Whittenbury



John Wilkinson

Hyphomicrobium



Sticks to surfaces; grows only on C1 and C2 compounds. Reproduce by budding

Grow on methanol and methylated amines but not methane

Hirsch & Conti, Harder, Dow, Attwood.
Quayle (assimilation pathways)



Hans van Dijken and Wim Harder 1973
Hyphomicrobium, Yeast and Growth
yields of methylotrophs

Methylotrophic Yeasts

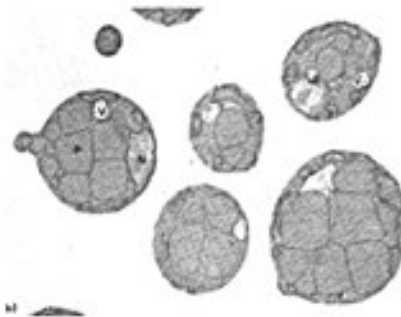
By contrast with bacteria, the methylotrophic yeasts are not a special group; they are in the usual genera of yeasts [*Hansenula*, *Candida*, *Pichia* and *Torulopsis*]. Only 7/39 genera grow methylotrophically. They grow only on methanol (not methylamines or methane). Again, by contrast with bacteria they all share the same assimilation pathways.

They oxidise methanol by a flavoprotein alcohol oxidase coupled to catalase which is present in crystalline form in peroxisomes and which yields no energy. Almost all bacteria use an energy-yielding quinoprotein methanol dehydrogenase. all

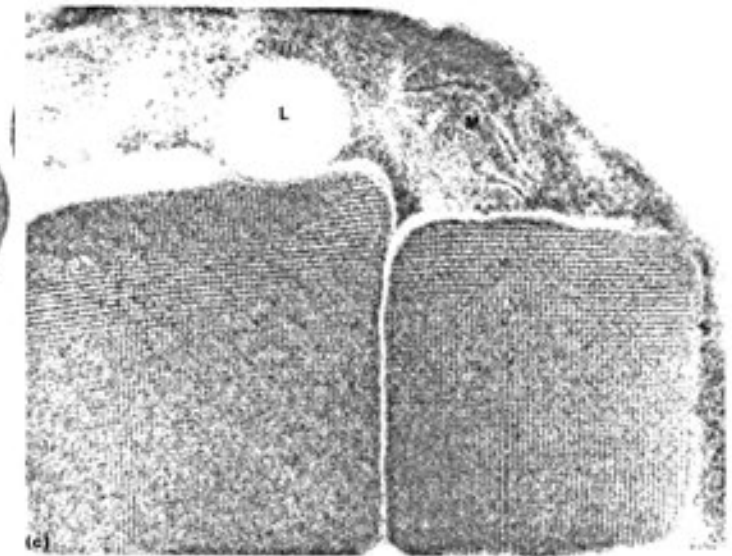
1969-1975

Ogata, Tani, Sahm, van
Dijken, Harder,
Quayle (assimilation)

Peroxisomes in methanol-oxidising yeast



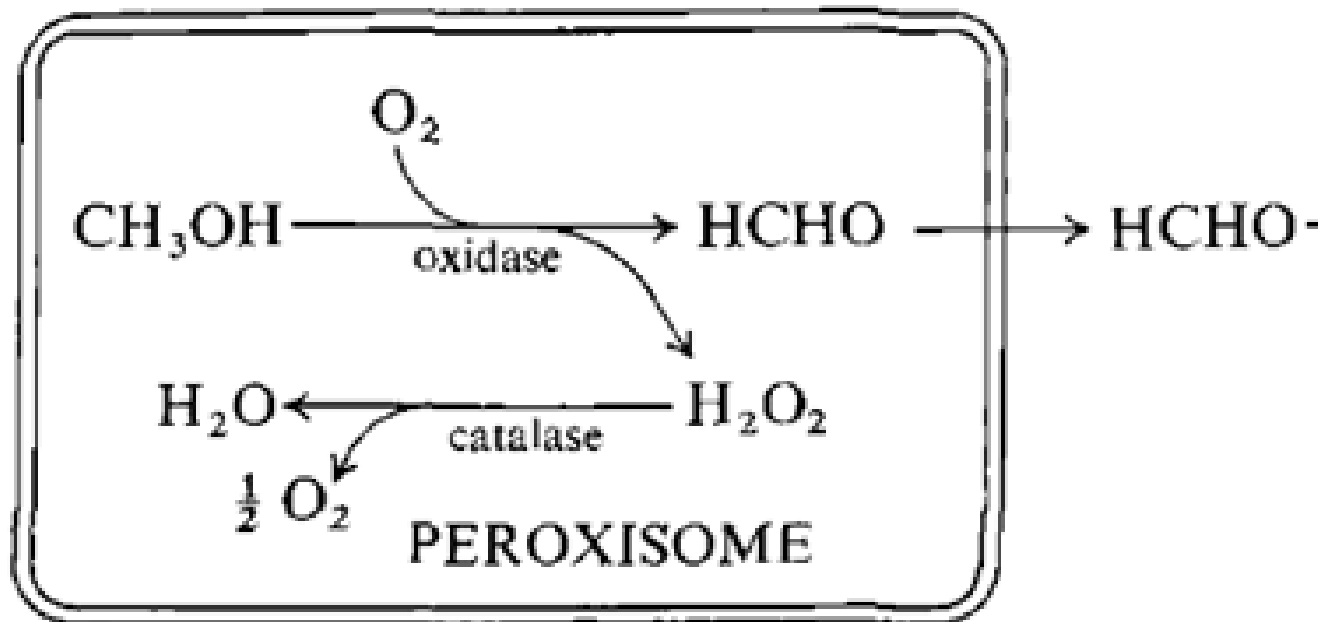
The oxidase is produced in very large amounts and transcription of the gene has a very strong promoter; it is now used in genetic engineering for production of large amounts of cloned Eucaryotic proteins.



Crystals of the flavoprotein alcohol oxidase in yeast

Methanol oxidation in methylotrophic yeast

This is catalysed by non-specific flavoprotein called alcohol oxidase
This is in a peroxisome with catalase



The enzyme is used as a source of hydrogen peroxide –
for wound disinfection and also used in washing powder



J. Guis Kuenen and Professor Kato (Tottori and Kyoto)



Friends from Yamaguchi, Japan: Matsushita, Uragami (?), Ameyama, Adachi

The main 'groups' of bacterial methylotrophs

Methanotrophs. Vast majority are obligate. (Recent important exceptions)

All have internal membranes and produce spores. Gram negative.

Type I (eg *Methylococcus*, *Methylomonas*) use RuMP pathway

Type II (eg *Methylosinus*, *Methylocystis*) use Serine pathway

Methylotrophs not using methane. None have internal membranes or produce spores. Vast majority are Gram negative.

Obligate methylotrophs Grow on methanol and/or methylamine. Gram negative, motile, non-sporing. Use RuMP pathway. Eg *Methylophilus*

Facultative methylotrophs. Usually grow on methanol/methylamine; use serine pathway

Pink facultative methylotrophs. Motile, single polar flagellum. Red carotenoids similar to photosynthetic bacteria. Wide range of C substrates but few carbohydrates. *Methylobacterium*

Non-pigmented facultative 'pseudomonads'. No typical pseudomonas sp use C1 compounds.

Pseudomonas aminovorans. Use serine pathway (icl⁺ variant)

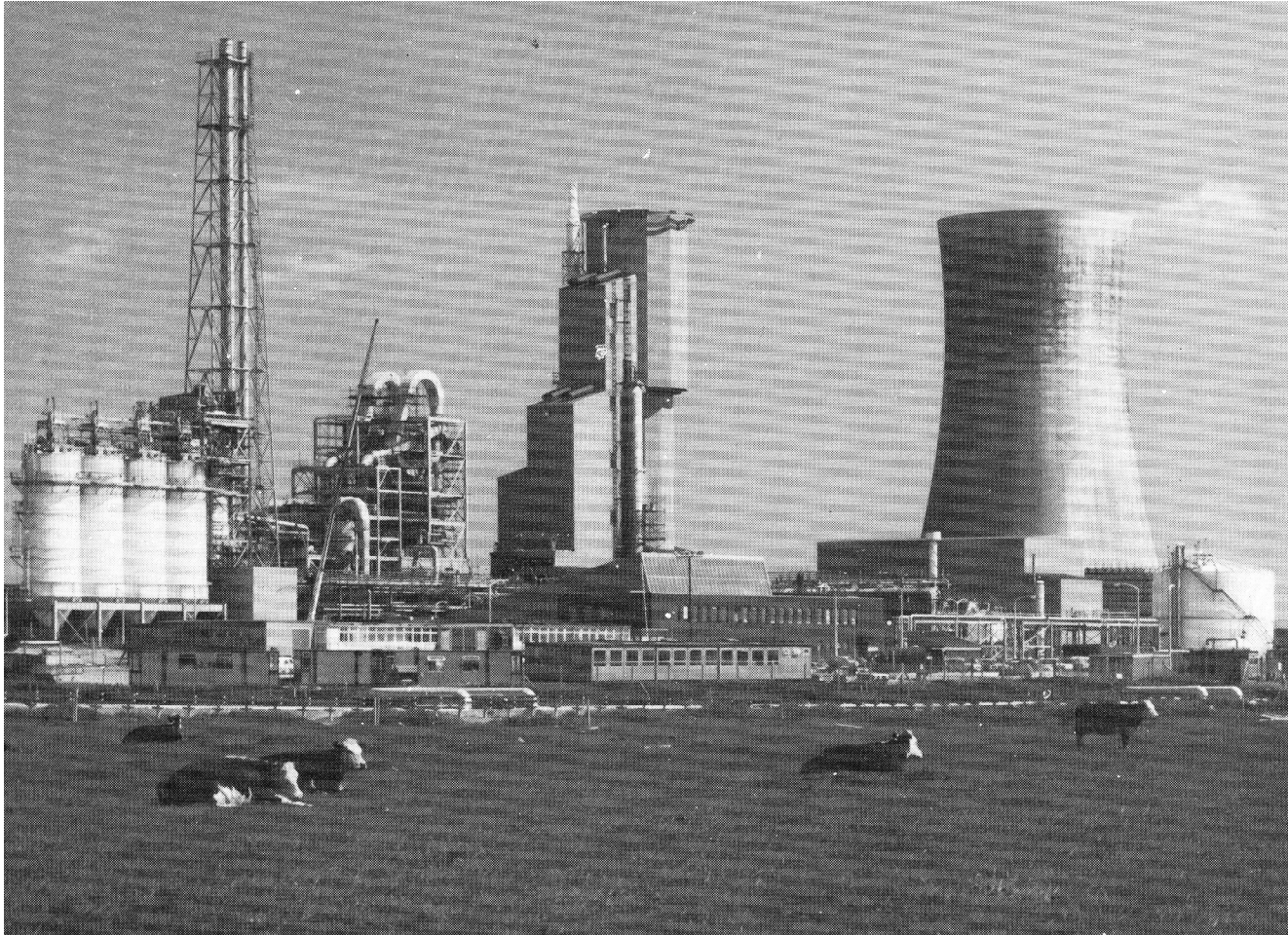
Gram-negative or variable non-motile rods. Some only use methylated amines. A mixed group. Eg *Arthrobacter*.

Facultative autotrophs or phototrophs Use RuBP pathway (Calvin cycle). *Paracoccus denitrificans* (can use hydrogen); *Rhodopseudomonas*

Gram positive facultative methylotrophs Very rare. *Bacillus*, *Streptomyces*

Hyphomicrobia Restricted facultative methylotrophs. Stalked. Budding. Serine pathway

The ICI 'Pruteen' plant at Billingham, UK 1980



The centre tower is the 1.5 million litre fermenter

It contains about 100,000,000,000,000,000,000 methylotrophs
10% of their soluble protein is Methanol dehydrogenase

The Methylootrophs and their biochemistry as was known up to 1981 is covered comprehensively in

The Biochemistry of Methylootrophs by Chris Anthony

It can be downloaded complete and free as a pdf file from my website

www.chris-anthony.co.uk

On this page: <http://www.chris-anthony.co.uk/methylootrophs.html>

The Biochemistry of Methylotrophs 1982

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C. ANTHONY

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England*

Chapters 12

Text pages 350

Index 48 pages

Tables 60

Figures 57

References 1300

1982



ACADEMIC PRESS

A Subsidiary of Harcourt Brace Jovanovich, Publishers

London · New York
Paris · San Diego · San Francisco
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Methylotrophic bacteria
Ribulose biphosphate pathway
Ribulose monophosphate cycle
The Serine pathway
The TCA cycle and growth
on multicarbon compounds
Oxidation of methane, methanol,
formaldehyde and formate
Oxidation of methylated amines
Electron transport and
energy transduction
Growth yields and bioenergetics
Methylotrophic yeasts
Methanogens and
methanogenesis
Commercial exploitation

A CD version is available and the complete book
is on my website: chris-anthony.co.uk

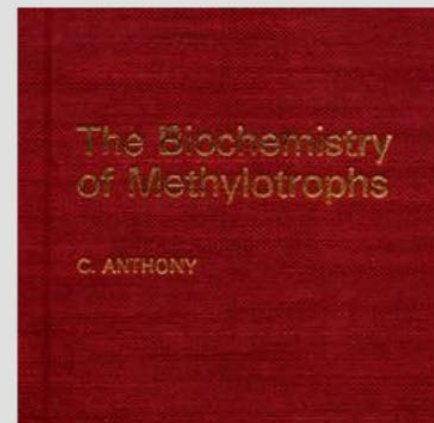
The Biochemistry of Methylootrophs

C. Anthony (1982) Academic Press, London

This textbook is no longer in print.

It contains 350 pages, 60 pages of Tables, 60 pages of Figures, a 40 page index and about 1000 references [every methylootroph reference up to 1981]. Copyright exclusive to author. Contents are summarised [below](#).

I have produced a CD of the book with all pages in both jpg and pdf formats which can be searched in Acrobat Reader. A copy of the CD is available [£25 or \$50].
Email: C.Anthony@soton.ac.uk



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Dedicated to K. G. Wiles, L. J. Zatman and J. R. Quayle, FRS

Foreword by Professor J.R. Quayle FRS

There are two kinds of organism which stand out as the biosynthetic virtuosi of the living world—the autotrophs and the methylootrophs. The autotrophs are organisms which can synthesize all their cell constituents from carbon dioxide as sole carbon source; methylootrophs can perform such total biosynthesis from reduced carbon compounds containing one or more carbon atoms but containing no carbon-carbon bonds. *Autotrophs have long been the subject of*

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