Background: Dr. Michael Köpke

- **PhD in Microbiology and Biotechnology** from Ulm University, Germany

- **10 years of experience** in Microbiology with a broad range of organisms
  - *Clostridium sp.*, *E. coli*, *Acetobacterium*, *Moorella*, *Lactococcus*, etc.

- **Publications in high impact journals and books**
  - Köpke et al. (2011) *Applied Environmental Microbiology* 77: 5467-75

- **Biology team leader at LanzaTech**

- **World leading experts as advisors**
  - Expertise in microbiology as well as physiology and ecology of bacteria

---

Prof. Dr. Dr. h.c. mult. Rudolf K. Thauer  
(Head of Emeritus Group at MPI Marburg, Germany)  
Prof. Dr. Ian Maddox  
(Academic Director of SEAT at Massey University)  
Prof. Dr. Peter Dürre  
(Director of Institute for Microbiology and Biotechnology at Ulm University, Germany)
**Clostridium magnum**

- Common bacteria, not associated with any adverse risks

- **Well characterized**
  - Described by Schink in 1984\(^a\), topic of several published studies\(^b\)
  - WHO Risk Group 1 (**No or low individual and community risk**) \(^c-e\)
    - Lowest rating, same as Baker’s yeast
    - A microorganism that is unlikely to cause human, plant or animal disease

- **Wide range of natural environments found to date**
  - Isolated of anoxic freshwater creek sediments (Germany, USA)\(^a,f\)
  - Isolated and detected of anoxic sludge from sewage plants (Germany, Korea)\(^a,g\)
  - Detected in anoxic **paper mill environment** (Finland)\(^h\)
  - Detected in **soil** of harvested potato plots (USA)\(^i\)
  - Detected in whey permeate **wastewater** (Korea)\(^j\)

- **Homoacetogenic and strict anaerobic** *Clostridium*

---

\(^e\) ABSA: [http://www.absa.org/riskgroups/index.html](http://www.absa.org/riskgroups/index.html)
Acetogenesis

• Ancient biochemical pathway with major impact in global carbon cycle

• **One of oldest existing pathways on earth**
  • Acetogens are characterized by using the reductive acetyl-CoA pathway with its unique enzyme complex Carbon monoxide dehydrogenase/Acetyl-CoA synthase<sup>a,b</sup>
  • This biochemical pathway is speculated to be the first biochemical pathway existing on earth, emerged millions of years ago<sup>c,d</sup>

• **Global impact**
  • ** Widely distributed<sup>a,b</sup>:** To date, over 100 species from over 20 different genera have been isolated to date from a variety of habitats (e.g. soil, sediments, sludge, intestinal tracts of animals and humans, hot springs) all over the globe, **including New Zealand<sup>e,f</sup>**
  • **Key role in global acetate cycle<sup>a,b</sup>:** It has been estimated that **10 trillion kg of acetate are synthesized per year in sediments** by acetogenesis<sup>g</sup>. Likewise, an estimated **10 trillion kg of acetate are produced annually via acetogenesis in the hindgut of termites<sup>h</sup>** and **100 billion kg of acetate in the human colon<sup>i-l</sup>**

---

<sup>f</sup> BioDiscovery NZ Ltd. (2008) Identification of *Clostridium autoethanogenum* in the New Zealand environment. Research report 04/06/2008
Anaerobic Lifestyle

• Acetogens are unable to survive in our atmosphere (21% oxygen)

• *Clostridium magnum dies at low oxygen concentrations*
  • Karnholz et al. tested effect of oxygen on *Clostridium magnum*, and found that growth was inhibited in presence of 0.5% oxygen (the lowest concentration tested), while cell death occurred immediately at concentrations as low as 1-2% oxygen

• Key enzymes are inactivated by oxygen
  • The reductive acetyl-CoA pathway is speculated to emerged long before oxygen accumulated in the atmosphere and most enzymes contain iron-sulfur-clusters
  • Key enzymes Carbon monoxide dehydrogenase/Acetyl-CoA synthase, Formate dehydrogenase and Pyruvate:Ferredoxin oxidoreductase are among the most oxygen-sensitive enzymes known

---


---

The initial concentrations (percentages by volume) of O2 in the headspaces of culture tubes were as follows: 0 (○), 0.5 (○), 1.0 (♦), and 2.0 (♢) (B); source: Karnholz et al., 2002

---

**Vol. 68, No. 2**

Tolerance and Metabolic Response of Acetogenic Bacteria toward Oxygen

Arno Karnholz, Kirsten Küsel, Anita Gößner, Andreas Schramm, and Harold L. Drake

Department of Ecological Microbiology, BITOEk, University of Bayreuth, 95440 Bayreuth, Germany

Received 27 July 2001/Accepted 13 November 2001
Growth conditions

- Limited range of conditions that allow growth

- Substrates
  - Limited substrate range, only few sugars and 2,3-butanediol allow growth (see table)\(^a\)
  - Later shown to be able to grow on gases CO\(_2\)/H\(_2\), but require presence of additional nutrients (e.g. yeast extract)\(^b\)

- Products
  - Acetate as sole fermentation end-product on all substrates\(^a,b\)

- Growth conditions
  - Needs an reduced environment\(^a\)
  - Temperature range: 15-45 °C (optimum at 30-32 °C)\(^a\)
  - Narrow pH range: pH 6.0-7.5 (optimum at 7.0)\(^a\)

- Inhibitors
  - Unable to grow in 1 % salt or more\(^a\)
    (seawater has an average of 3.5 % salt)

---
Sporulation

• LanzaTech process selects for Asporogenous strains

• Continuous fermentation selects for asporogenous strains:
  • As shown for Clostridia species by Meinecke et al., 1984a


  Selection of an Asporogenous Strain of Clostridium acetobutylicum in Continuous Culture Under Phosphate Limitation

  BEATRIX MEINECKE, HUBERT BAHL, AND GERHARD GOTTSCHALK*

  Institut für Mikrobiologie der Universität Göttingen, Grisebachstraße 8, D-3400 Göttingen, Federal Republic of Germany

  Received 22 May 1984/Accepted 23 August 1984

• Minimal fermentation conditions and media:
  • Carbon monoxide (CO) is toxic to most living organisms

• No sugar or any other complex substrates (e.g. yeast extract) in media

  Clostridium magnum has been reported to form spores on sugarsb, but sporulation on gaseous substrates has never been reported in the literaturec or observed by LanzaTech or in literatureb

---

Growth in pure culture

• **Clostridium magnum** inhibits itself in pure culture

• **Rising acetate levels inhibit growth**
  • Acetate (or acetic acid) is known to inhibit acetogenesis and bacterial growth, leading to cell death at small concentrations\(^a,b\)
  • **Clostridium magnum** produces acetate as sole metabolic end-product\(^c,d\)
  • As a result, acetogenesis and growth are inhibited and cell death occurs within a few hours when acetate is not removed\(^a,b,d\)
  • Bomar et al., 1991 demonstrate growth of **Clostridium magnum** in different growth media and CO\(_2\) and H\(_2\) as substrate. Growth stops within a few hours and cell death occurs


\(^d\) Bomar et al. (1991) Litotrophic growth and hydrogen metabolism by **Clostridium magnum**. *FEMS Microbiol. Lett.* 83: 347-50

Fig. 3. Growth and hydrogen formation by **Clostridium magnum** strain WoBdP1 with 5 mM glucose in different growth media. (●) cell density and (○) hydrogen formation in the presence of 5 mM NH\(_4\)Cl; (■) cell density and (□) hydrogen formation under N\(_2\)/CO\(_2\) in the absence of bound nitrogen; (▲) cell density and (△) hydrogen formation under argon/CO\(_2\) in the absence of bound or gaseous nitrogen.

source: Bomar et al., 1991\(^d\)
Growth in community

• Acetogens like *Clostridium magnum* exist and can be found in many different habitats but are unable to dominate bacterial communities or environments due to simple energetic reasons

• While *Clostridium magnum* is inhibited by the acetate produced, it serves as carbon and energy source to many other organisms\(^a\)

• Thermodynamics of bacterial communities:
  • Organisms using the electron acceptor with the highest Gibbs free energy dominate over groups using less favorable electron acceptors\(^b-g\)
  • Competing processes of sulfate reduction, methanogenesis are thermodynamically more favorable than acetogenesis\(^a,d-g\)
    *Sulfate reduction: 4H\(_2\) + SO\(_4^{2-}\) + H\(^+\) → HS\(^-\) + 4H\(_2\)O (\(\Delta G'_o = -152.2\) kJ)*
    *Methanogenesis: 4H\(_2\) + HCO\(_3^-\) + H\(^+\) → CH\(_4\) + 3H\(_2\)O (\(\Delta G'_o = -135.6\) kJ)*
    *Acetogenesis: 4H\(_2\) + 2HCO\(_3^-\) + H\(^+\) → CH\(_3\)COO\(^-\) + 4H\(_2\)O (\(\Delta G'_o = -104.6\) kJ)*


Fig. 20. Cross-section of a soil aggregate showing a hypothetical anoxic core and possible trophic links between acetate and other redox processes during the oxidation of soil organic carbon to CO\(_2\). Modified from Drake et al. (1997). Source: Oren, 2012*\(^g\)
## Growth in community Example 1

- **Clostridium magnum** is present but unable to dominate a complex community

- Study by Lee et al., 2008
- Investigates shifts in bacterial and archeal communities shifts in anaerobic digester

### Table of Band Nearest species and taxon:

<table>
<thead>
<tr>
<th>Band</th>
<th>Nearest species and taxon</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>Uncultured Fuscibacteria</td>
</tr>
<tr>
<td>W2</td>
<td>Clostridium tertium</td>
</tr>
<tr>
<td>W3</td>
<td><em>Trichococcus flocculiformis</em></td>
</tr>
<tr>
<td>W4</td>
<td>Aeromonas caviae</td>
</tr>
<tr>
<td>W5</td>
<td>Clostridium acetobutylicum</td>
</tr>
<tr>
<td>W6</td>
<td><em>C. acetobutylicum</em></td>
</tr>
<tr>
<td>W7</td>
<td><em>C. acetobutylicum</em></td>
</tr>
<tr>
<td>W8</td>
<td><strong>Clostridium magnum</strong></td>
</tr>
<tr>
<td>W9</td>
<td><em>Streptococcus bovis</em></td>
</tr>
<tr>
<td>W10</td>
<td><strong>C. magnum</strong></td>
</tr>
<tr>
<td>W11</td>
<td>Anaeroflobium agaric</td>
</tr>
<tr>
<td>W12</td>
<td>Uncultured bacterium RB016</td>
</tr>
<tr>
<td>W13</td>
<td>Uncultured bacterium</td>
</tr>
<tr>
<td>W14</td>
<td>Uncultured bacterium IA-5</td>
</tr>
<tr>
<td>W15</td>
<td>Uncultured soil bacterium</td>
</tr>
<tr>
<td>W16</td>
<td>Uncultured bacterium IB-27</td>
</tr>
<tr>
<td>W17</td>
<td>Clostridium stakielmii</td>
</tr>
<tr>
<td>W18</td>
<td>Uncultured bacterium RB016</td>
</tr>
<tr>
<td>W19</td>
<td>Clostridium sp. 13A1</td>
</tr>
<tr>
<td>W20</td>
<td>Uncultured bacterium E3</td>
</tr>
<tr>
<td>W21</td>
<td>Uncultured bacterium E16</td>
</tr>
<tr>
<td>W22</td>
<td>Clostridium sp. 13A1</td>
</tr>
</tbody>
</table>

### Figure 4: Bacterial DGGE profiles of the PCR products amplified with 16S rRNA gene primers.

Source: Lee et al., 2008

---

Growth in community Example 2

- *Clostridium magnum* is present but unable to dominate a complex community

- Study by Kim et al., 2011
- Investigates shifts in bacterial and archeal communities shifts in anaerobic digester

---

[source: Kim et al., 2011]

*Kim J. et al. (2011) Common key acidogen populations in anaerobic reactors treating different wastewaters: Molecular identification and quantitative monitoring. Water Res. 45: 2539-49*
Summary

- *Clostridium magnum* has been well described in several studies and is not associated to any adverse risks.

- Acetogens like *Clostridium magnum* are widely distributed all over the globe (including New Zealand) as they have a major role on the global carbon cycle.

- Anaerobic organisms like *Clostridium magnum* are unable to survive in our oxygen-rich atmosphere.

- While *Clostridium magnum* is described to sporulate, sporulation on gaseous substrates has never been reported and the LanzaTech process selects for Asporogenous strains.

- While acetogens like *Clostridium magnum* exist and can be found in many different habitats (e.g. sediments, soil, sludge, intestinal tracts of animals and humans, hot springs), they are unable to dominate bacterial communities or whole environments due to simple energetic reasons and self inhibition.