# Background: Dr. Michael Köpke

LanzaTech

- 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**
    - **Köpke *et al.* (2011) *Current Opinion in Biotechnology* 23: 320-5**
    - **Köpke *et al.* (2011) *Biofuel Production* (ISBN 978-953-307-478-8)**
    - **Köpke *et al.* (2010) *Proceedings of the National Academy of Sciences* 107: 13087-92**
    - **Köpke & Dürre (2010) *Handbook of biofuel production* (ISBN 978-1-84569-679-5)**
    - **Köpke *et al.* (2009) *Laborwelt* 6: 16-17**
    - **Noack *et al.* (2008) *New Research on Biofuels* (ISBN 978-1-60456-828-8)**
- 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*

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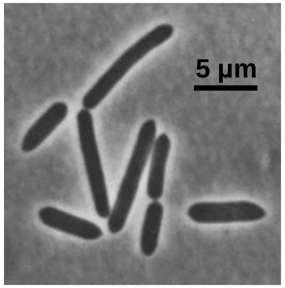



source: Schink et al., 1984[a]

- **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***

**Taxonomy[k]**
- **Superkingdom: *Bacteria***
- **Phylum: *Firmicutes***
- **Class: *Clostridia***
- **Order: *Clostridiales***
- **Family: *Clostridiacaea***
- **Genus: *Clostridium***
- **Species: *magnum***

[a] Schink B. (1984) *Clostridium magnum* sp. nov., a non-autotrophic homoacetogenic bacterium. *Arch. Microbiol.* 137: 250-5
[b] PubMed: http://www.ncbi.nlm.nih.gov/pubmed?term=clostridium%20magnum
[c] DSMZ: http://www.dsmz.de/catalogues/details/culture/DSM-2767.html?tx_dsmzresources_pi5[returnPid]=304
[d] ATCC: http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=49199&Template=bacteria
[e] ABSA: http://www.absa.org/riskgroups/index.html
[f] Tangalos G. *et al.* (2007) Microbiological and iron-isotopic evidence for dissimilatory iron reduction in reservoir sediment near Iron mountain, California. GSA Denver Annual Meeting 2007: http://gsa.confex.com/gsa/2007AM/finalprogram/abstract_124771.htm
[g] Lee C. *et al.* (2008) Monitoring bacterial and archeal community shifts in a mesophilic anaerobic batch reactor treating a high strength organic wastewater. *FEMS Microbiol. Ecol.* 65: 544-54
[h] Suihko M.-L. *et al.* (2005) Occurrence and molecular characterization of cultivable mesophilic and thermophilic obligate anaerobic bacteria isolated from paper mills. *Sys. Appl. Microbiol.* 28: 555-61
[i] Luo Y. *et al.* (2008) Organic loading rates affect composition of soil-derived bacterial communities during continious, fermentative biohydrogen production. *Int. J. Hydrogen Energy* 33: 6566-76
[j] 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
[k] NCBI: http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=33954&lvl=3&keep=1&srchmode=1&unlock&lin=f

# Acetogenesis

LanzaTech

- **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**[a,b]
  - **This biochemical pathway is speculated to be the first biochemical pathway existing on earth, emerged millions of years ago**[c,d]

- **Global impact**
  - **Widely distributed**[a,b]**: 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**[e,f]
  - **Key role in global acetate cycle**[a,b]**: It has been estimated that 10 trillion kg of acetate are synthesized per year in sediments by acetogenesis**[g]**. Likewise, an estimated 10 trillion kg of acetate are produced annually via acetogenesis in the hindgut of termites**[h] **and 100 billion kg of acetate in the human colon**[i-l]

[a] Drake H. L., *et al.* (2008) Old acetogens, New light. *Ann. N. Y. Acad. Sci.* 1125: 100-28
[b] Drake H. L., *et al.* (2006) Acetogenic prokaryotes. In: Dworkin M. *et al.* (Eds.) *The Prokaryotes*, 3rd Ed., Vol. 2 (Ecophysiology and Biochemistry). Springer: 354-420
[c] Russell M. J. and Martin W. (2004) The rocky roots of the acetyl-CoA pathway. *TRENDS in Biochem. Sci.* 29: 358-63
[d] Martin W. F. (2012) Hydrogen, metals, bifurcating electrons, and proton gradients: The early evolution of biological energy conservation. *FEBS Lett.*: Epub Ahead of print
[e] Patel B. K. C., *et al.* (1987) *Clostridium fervidus* sp. nov., a new chemoorganotrophic acetogenic thermophile. *Int. J. Syst. Evol. Microbiol.* 2: 123-6
[f] BioDiscovery NZ Ltd. (2008) Identification of *Clostridium autoethanogenum* in the New Zealand environment. Research report 04/06/2008
[g] Wood H. G. and Ljungdahl L. G. (1991) Autotrophic character of acetogenic bacteria. In: Shively J. M. and Barton L. L. (Eds.) *Variations in Autotrophic Life.* Academic Press: 201-50
[h] Breznak J. A. and Kane M. D. (1990) Microbial $H_2/CO_2$ acetogenesis in animal guts: nature and nutritional significance. *FEMS Microbiol Rev.* 7: 309-13
[i] Lajoie S. F., et al. (1988) Acetate production from hydrogen and [13C]carbon dioxide by the microflora of human feces. *Appl. Environ. Microbiol.* 54: 2723–27
[j] Wolin M. J. and Miller T. L. (1994) Acetogenesis from $CO_2$ in the human colonic ecosystem. In: Drake H. L. (Ed.) *Acetogenesis.* Chapman and Hall: 365–85
[k] Doré, J., *et al.* (1995) Enumeration of $H_2$-utilizing methanogenic archaea, acetogenic and sulfate-reducing bacteria from human feces. *FEMS Microbiol. Ecol.* 17: 279–84
[l] Bernalier A., et al. (1996) Diversity of $H_2/CO_2$-utilizing acetogenic bacteria from feces of non-methane-producing humans. *Curr. Microbiol.* 33: 94–99

# Anaerobic Lifestyle

LanzaTech

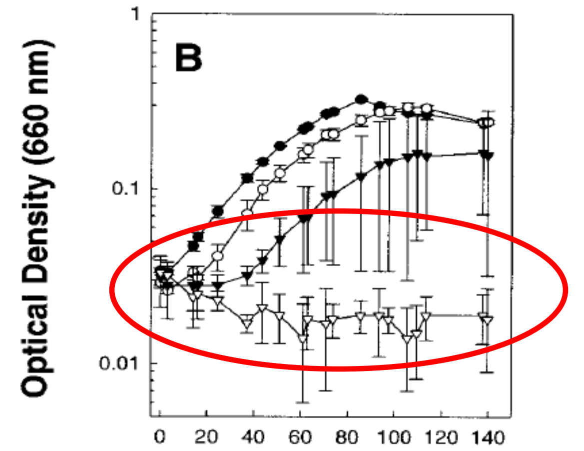
- **Acetogens are unable to survive in our atmosphere (21 % oxygen)**
- ***Clostridium magnum* dies at low oxygen concentrations**
  - **Karnholz *et al.*[a] tested effect of oxygen on *Clostridium magnum*, and found that growth was inhibited in presence of 0.5 % oxygen (the lowest concentration tested), while cell death occurred immediately at concentrations as low as 1-2 % oxygen**

Applied and Environmental Microbiology, Feb. 2002, p. 1005–1009 Vol. 68, No. 2
0099-2240/02/$04.00+0 DOI: 10.1128/AEM.68.2.1005–1009.2002


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




The initial concentrations (percentages by volume) of $O_2$ in the headspaces of culture tubes were as follows: 0 (●), 0.5 (○), 1.0 (▼), and 2.0 (▽) (B);

source: Karnholz et al., 2002[a]

- **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[b]**
  - **Key enzymes Carbon monoxide dehydrogenase/Acetyl-CoA synthase[c], Formate dehydrogenase[d] and Pyruvate:Ferredoxin oxidoreductase[e] are among the most oxygen-sensitive enzymes known**

[a] Karnholz A.*et al.* (2002) Tolerance and metabolic response of acetogenic bacteria toward oxygen. *Appl. Environ. Microbiol.* 68: 1005-9.
[b] Russell M. J. and Martin W. (2004) The rocky roots of the acetyl-CoA pathway. *TRENDS in Biochem. Sci.* 29: 358-63.
[c] Ragsdale S. W., et al. (1983) 13C and 61Ni isotope substitution confirm the presence of a nickel(III)-carbon species in acetogenic CO dehydrogenases. *Biochem. Biophys. Res. Commun.* 115:658–665.
[d] Drake H. L., *et al.* (2006) Acetogenic prokaryotes. In: Dworkin M. *et al.* (Eds.) *The Prokaryotes*, 3rd Ed., Vol. 2 (Ecophysiology and Biochemistry). Springer: 354-420.
[e] Meinecke B. (1989) Purification and characterization of the pyruvate-ferredoxin oxidoreductase from *Clostridium acetobutylicum. Arch. Microbiol.* 152: 244-50.

# Growth conditions

LanzaTech

- **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)**

| Substrate | Strain Wo Bd P1 |
|---|---|
| $H_2/CO_2$ | – |
| Formate | – |
| Methanol | – |
| Ethylene glycol | – |
| Ethanol | – |
| Serine | – |
| Lactate | – |
| Pyruvate | ± |
| Malate | + |
| Fumarate | – |
| Citrate | + |
| Glycerate | – |
| Glycerol | – |
| Glutamate | – |
| Fructose | + |
| Glucose | + |
| Arabinose | – |
| Xylose | + |
| Ribose | – |
| 2,3-Butanediol | + |
| Acetoin | + |

source: Schink et al., 1984[b]

[a] Schink B. (1984) *Clostridium magnum* sp. nov., a non-autotrophic homoacetogenic bacterium. *Arch. Microbiol.* 137: 250-5
[b] Bomar et al. (1991) Litotrophic growth and hydrogen metabolism by *Clostridium magnum. FEMS Microbiol. Lett.* 83: 347-50

# Sporulation

LanzaTech

- **LanzaTech process selects for Asporogenous strains**

- **Continuous fermentation selects for asporogenous strains:**
  - **As shown for Clostridia species by Meinecke et al., 1984[a]**

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1984, p. 1064–1065 Vol. 48, No. 5
0099-2240/84/111064-02$02.00/0


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, Grisebachstraβe 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 sugars[b],**
    **but sporulation on gaseous substrates has never been reported in the literature[c]**
    **or observed by LanzaTech or in literature[b]**

[a] Meinecke B., *et al.* (1984) Selection of an asporogenous strain of *Clostridium acetobutylicum* in Continious culture under phosphate limitation. *Appl. Environ. Microbiol.* 48: 1064-5
[b] Schink B. (1984) *Clostridium magnum* sp. nov., a non-autotrophic homoacetogenic bacterium. *Arch. Microbiol.* 137: 250-5
[b] Bomar et al. (1991) Litotrophic growth and hydrogen metabolism by *Clostridium magnum. FEMS Microbiol. Lett.* 83: 347-50

# Growth in pure culture

LanzaTech

- ***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 occures**

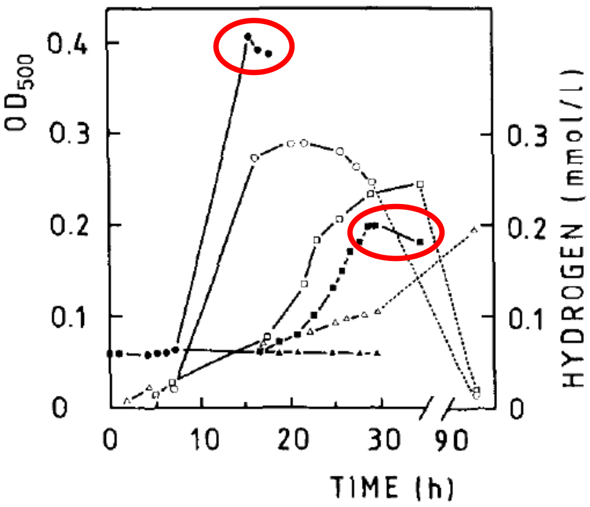



Fig. 3. Growth and hydrogen formation by *Clostridium magnum* strain WoBdP1 with 5 mM glucose in different growth media. (●) cell density and (○) hydrogen formation in the presence of 5 mM $NH_4Cl$; (■) cell density and (□) hydrogen formation under $N_2/CO_2$ in the absence of bound nitrogen; (▲) cell density and (△) hydrogen formation under argon/$CO_2$ in the absence of bound or gaseous nitrogen.

source: Bomar et al., 1991[d]

[a] Wang G. and Wang D. I. C. (1984) Elucidation of growth inhibition and acetic acid production by *Clostridium thermoaceticum. Appl. Environ. Microbiol.* 47: 294-8
[b] Baronofsky J. J., et al. (1984) Uncoupling by acetic acid limits growth of and acetogenesis by *Clostridium thermoaceticum. Appl. Environ. Microbiol. 48*: 1134-39
[c] Schink B. (1984) *Clostridium magnum* sp. nov., a non-autotrophic homoacetogenic bacterium. *Arch. Microbiol.* 137: 250-5
[d] Bomar et al. (1991) Litotrophic growth and hydrogen metabolism by *Clostridium magnum. FEMS Microbiol. Lett.* 83: 347-50

# Growth in community

LanzaTech

- **Acetogens like *Clostridium magnum* exist and can be found in many different habitates 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: $4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$ ($\Delta G'_O = -152.2$ kJ)**
    - **Methanogenesis: $4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$ ($\Delta G'_O = -135.6$ kJ)**
    - **Acetogenesis: $4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 4H_2O$ ($\Delta G'_O = -104.6$ kJ)**

    source: Oren, 2012[g]

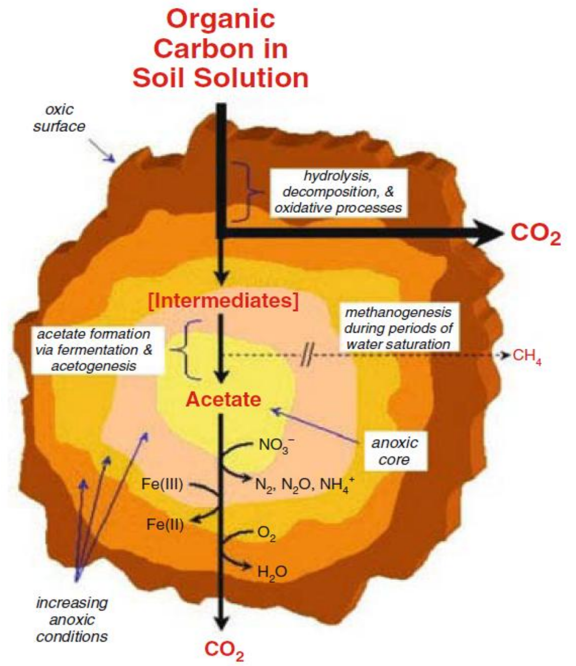



Fig. 20. Cross-section of a soil aggregate showing a hypothetical anoxic core and possible trophic links between acetate and other redox processes during the oxidation of soil organic carbon to $CO_2$. Modified from Drake et al. (1997).

source: Drake et al., 2006[a]

[a] Drake H. L., *et al.* (2006) Acetogenic prokaryotes. In: Dworkin M. *et al.* (Eds.) *The Prokaryotes*, 3rd Ed., Vol. 2 (Ecophysiology and Biochemistry). Springer: 354-420
[b] Cappenberg T. E. (1974) Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a fresh-water lake. II. Inhibition experiments. *Antonie Van Leeuwenhoek* 56: 1247-58
[c] Lovley D. R. and Goodwin S. (1988) Hydrogen concentrations as an indicator of the terminal electron-accepting reactions in aquatic sediments. *Geochim. Cosmochim. Acta* 52: 2993-3003
[d] Hoehler T. M., et al. (1999) Acetogenesis from $CO_2$ in ananoxic marine sediment. *Limnol. Oceanogr.* 44: 662-67
[e] Lever M. A., et al. (2010) Acetogenesis in deep subseafloor sediments of the Juan de Fuca ridge flank: A synthesis of geochemical, thermodynamic, and gene-based evidence. *Geomicrobiol. J.* 27: 183-211
[f] Lever M. A. (2012) Acetogenesis in the energy-starved deep biosphere – a paradox? *Front. Microbiol.* 2:284 (Epub)
[g] Oren A. (2012) There must be an acetogen somewhere. *Front Microbiol.* 3: 22 (Epub)

# Growth in community Example 1

LanzaTech

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

- **Study by Lee et al., 2008[a]**
- **Investigates shifts in bacterial and archeal communities shifts in anaerobic digestor**

RESEARCH ARTICLE

**Monitoring bacterial and archaeal community shifts in a mesophilic anaerobic batch reactor treating a high-strength organic wastewater**

Changsoo Lee[1], Jaai Kim[2], Seung Gu Shin[2] & Seokhwan Hwang[2]

[1]Microbial Ecology Laboratory, Department of Microbiology and Environmental Change Institute (ECI), National University of Ireland, Galway, Ireland; and [2]School of Environmental Science and Engineering, Pohang, Gyungbuk, Korea

| Band | Nearest species and taxon |
|---|---|
| W1 | Uncultured *Fusobacteria* |
| W2 | *Clostridium tertium* |
| W3 | *Trichococcus flocculiformis* |
| W4 | *Aeromonas caviae* |
| W5 | *Clostridium acetobutylicum* |
| W6 | *C. acetobutylicum* |
| W7 | *C. acetobutylicum* |
| W8 | *Clostridium magnum* |
| W9 | *Streptococcus bovis* |
| W10 | *C. magnum* |
| W11 | *Anaerofilum agile* |
| W12 | Uncultured bacterium RB016 |
| W13 | Uncultured bacterium |
| W14 | Uncultured bacterium IA-5 |
| W15 | Uncultured soil bacterium |
| W16 | Uncultured bacterium IIB-27 |
| W17 | *Clostridium sticklandii* |
| W18 | Uncultured bacterium RB016 |
| W19 | *Clostridium* sp. 13A1 |
| W20 | Uncultured bacterium E3 |
| W21 | Uncultured bacterium E16 |
| W22 | *Clostridium* sp. 13A1 |

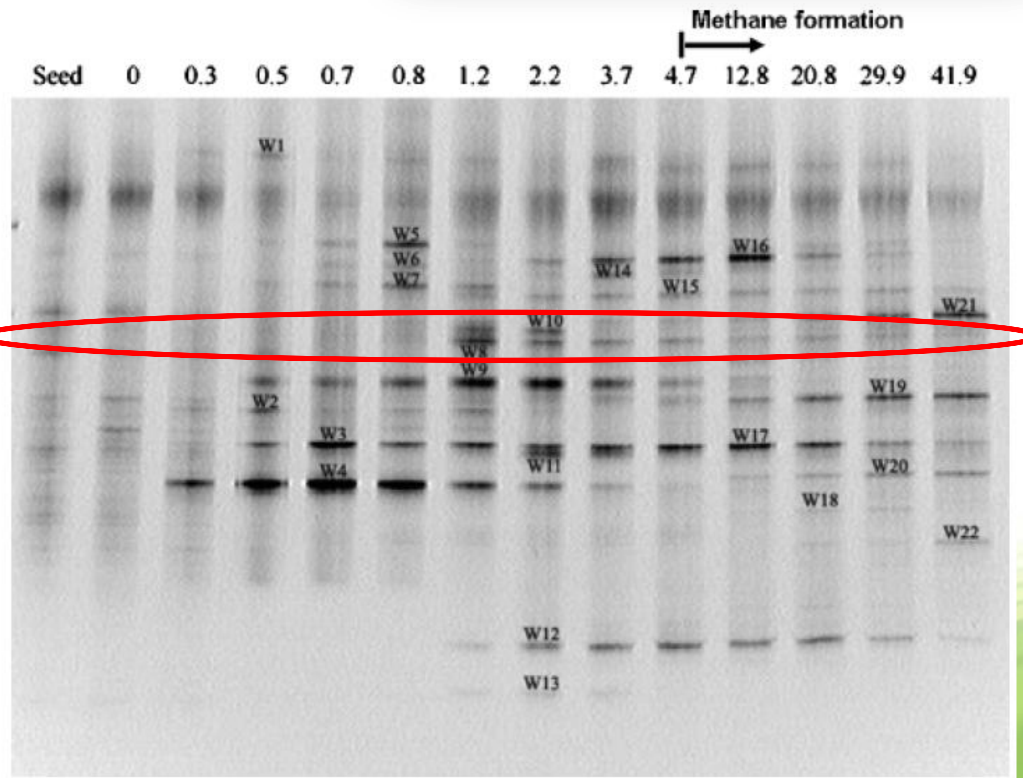



**Fig. 4.** Bacterial DGGE profiles of the PCR products amplified with 16S rRNA gene primers.

source: Lee et al., 2008[a]



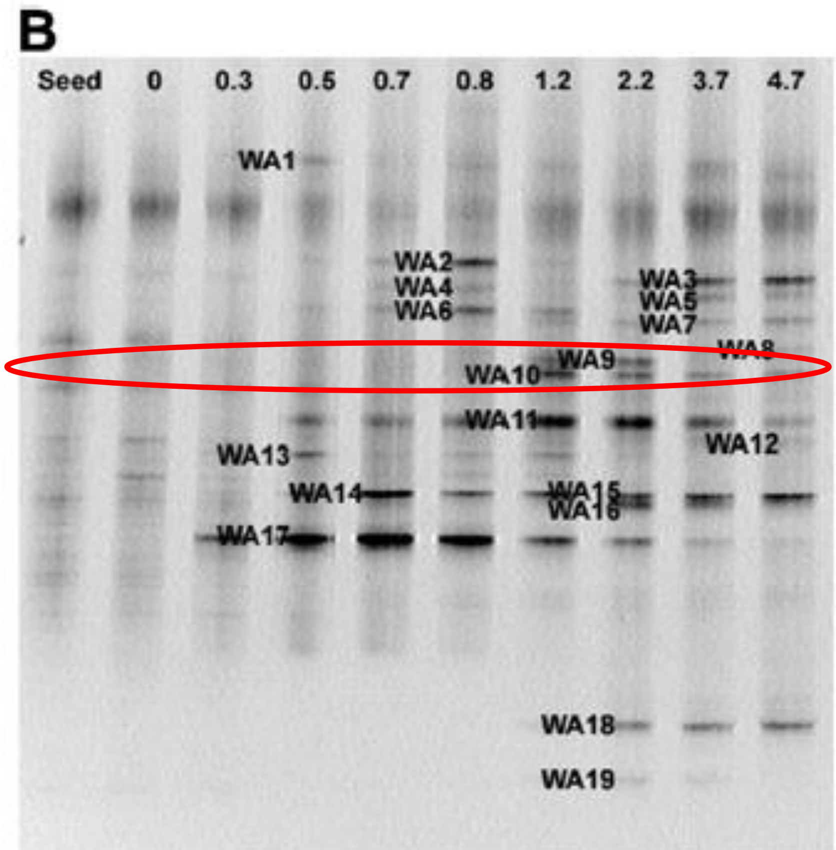
[a] Lee C. *et al.* (2008) Monitoring bacterial and archeal community shifts in a mesophilic anaerobic batch reactor treating a high strength organic wastewater. *FEMS Microbiol. Ecol.* 65: 544-54

# Growth in community Example 2

LanzaTech

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

- Study by Kim et al., 2011[a]
- Investigates shifts in bacterial and archeal communities shifts in anaerobic digestor




WATER RESEARCH 45 (2011) 2539–2549

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journal homepage: www.elsevier.com/locate/watres

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WATER RESEARCH

**Common key acidogen populations in anaerobic reactors treating different wastewaters: Molecular identification and quantitative monitoring**

*Jaai Kim*[a], *Seung Gu Shin*[b], *Gyuseong Han*[b], *Vincent O'Flaherty*[c], *Changsoo Lee*[a,*], *Seokhwan Hwang*[b,**]

[a] Division of Environmental and Water Resources Engineering (EWRE), School of Civil and Environmental Engineering, Nanyang Technological University, Singapore
[b] School of Environmental Science and Engineering, POSTECH, San 31, Hyoja-dong, Nam-gu, Pohang, Gyungbuk 790-784, Republic of Korea
[c] Microbial Ecology Laboratory, Department of Microbiology and Environmental Change Institute (ECI), National University of Ireland, Galway, Ireland

| Closest species and taxon (accession no.) | W-reactor |
|---|---|
| *Aeromonas hydrophila* (X87271) | WA17 |
| *Anaerofilum agile* (X98011) | WA16 |
| *Clostridium acetibutylicum* (X81021) | WA2 |
| *Clostridium magnum* (X77835) | WA9 |
| | WA10 |
| *Clostridium* sp. 13A1 (AY554421) | WA12 |
| *Clostridium sticklandii* (M26494) | WA15 |
| *Clostridium tertium* (AJ245413) | WA13 |
| *Streptococcus bovis* (AF429766) | WA11 |
| *Trichococcus flocculiformis* (AJ306611) | WA14 |
| Ubc E16 (AY426460) | WA8 |
| Ubc Galb35 (AY193168) | WA1 |
| Ubc IA-5 (AJ488076) | WA5 |
| Ubc M13 (DQ378233) | WA7 |
| Ubc mek63d03 (AY537432) | WA19 |
| Ubc RB016 (AB240286) | WA18 |

Ubc, uncultured bacterial clone.

source: Kim et al., 2011[a]



[a] 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

LanzaTech

- ***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**