

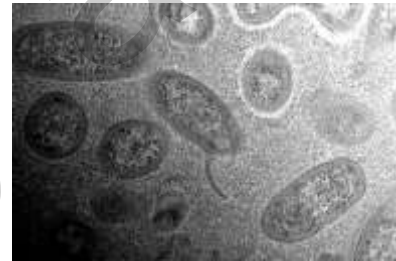
# Gut anaerobes in health and disease

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A mixed  
bowl of  
fruit



# Gut anaerobes

- **Zhernakova et al., Science 2016 - Dutch population-based Cohort:**  
*"Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity"*
- Most of the reads (97.6%) came from Bacteria; 2.2% were from *Archaea*, 0.2% from *Viruses*, and <0.01% from *Eukaryotes*.  
**(Bacteria: 99% anaerobes)**
- Gut microbiome (collective genomes)  $\approx$  gut anaerobiome
- Gut microbiota (collective microorganisms)  $\approx$  gut anaerobiota = gut anaerobes

# In health and disease

Implying an imbalance – disease

Which might be corrected to health?

Studies so far are mostly describing disease and health baseline composition in humans, e.g. the *Firmicutes/Bacteroidetes* ratio...

- At the phylum level, the abundances of dominant bacterial phyla *Firmicutes*(63.7%) and *Bacteroidetes* (8.1%)

(Zhernakova et al., Science 2016)



# MALDI-TOF

- Mainly used from the disease perspective
  - identification from source -> consider treatment
- Identification from blood consider -> source (normal habitat of pathogen) and treatment



# Gut anaerobes in health and disease

- Very exciting and hot topic
- Setting the scene
- Where does all this new knowledge come from?

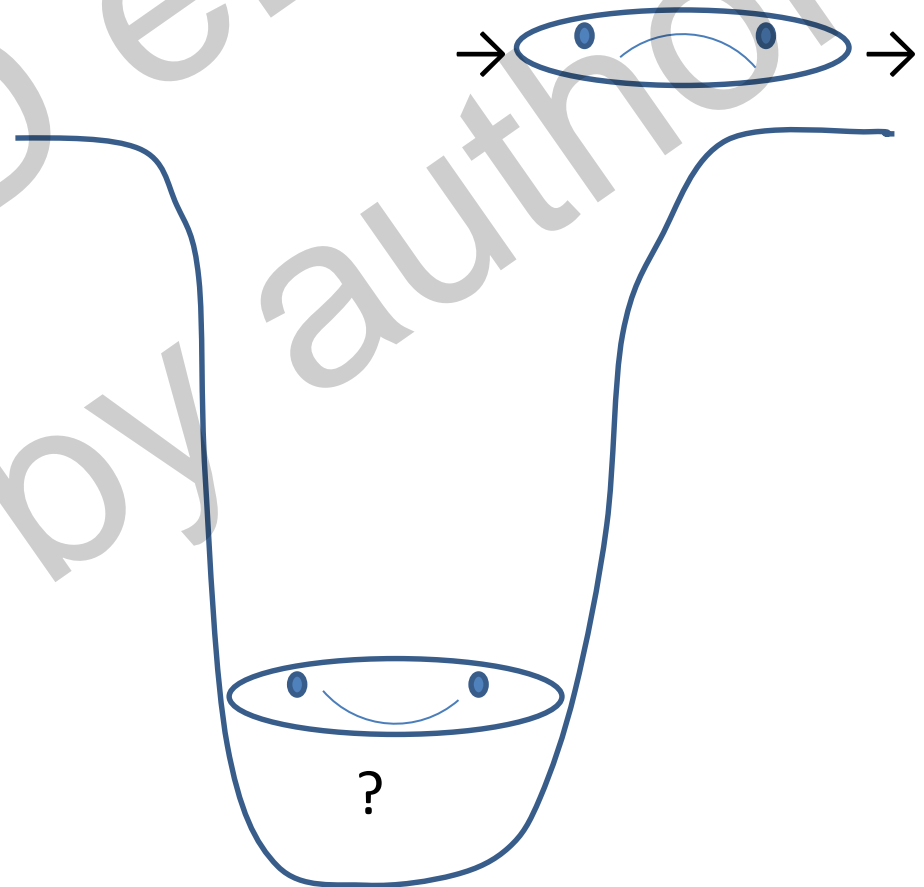
**DETOUR**



# Faecal samples as a surrogate?

What is happening at the bottom of the colonic crypts?

- Many studies are performed exclusively on faecal samples
- Described as the gut microbiome, although it is the faecal microbiome.



# Composition of the microbiota

## Composition depending on stool consistency and ...

... chocolate-type preference (an increased abundance of unclassified *Lachnospiraceae* were seen in participants with a preference for dark chocolate).

## Falony et al., Science 2016.

... *Akkermansia* abundance positively correlated with **time since previous relief**, but it was also negatively associated with insulin resistance risk factors such as BMI and blood triglyceride concentrations.

## Alternative Stool Chart

Type 1		
Type 2		
Type 3		
Type 4		
Type 5		
Type 6		
Type 7		



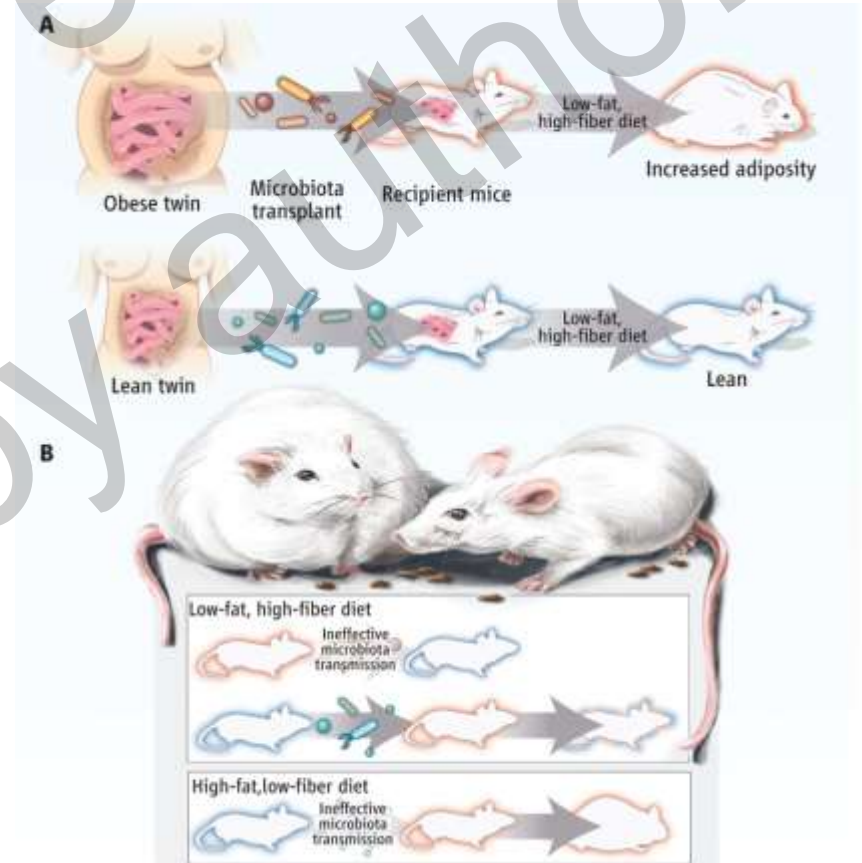
# Obesity

*Firmicutes/Bacteroidetes* ratio as being one of the key signatures of obesity

Walker and Parkhill, Science 2013

– comment on Ridaura et al, Science 2013

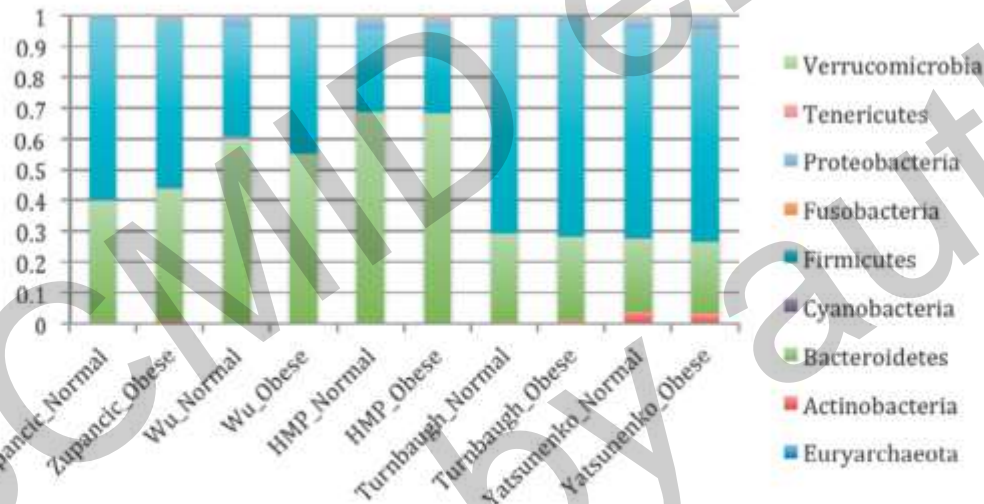
Analysis of the bacterial communities showed that Members of the Bacteroidetes phylum, particularly *Bacteroides* spp., could pass from the Lean mice and colonize the Obese mice, suggesting that these bacteria were largely responsible for protection against increased adiposity.





## Meta-analyses of human gut microbes associated with obesity and IBD

William A. Walters<sup>a</sup>, Zech Xu<sup>b</sup>, Rob Knight<sup>b,c,d,\*</sup>



**Fig. 1.** Relative abundance of phylum-level gut microbial taxa. Studies listed below are Zupancic [25], Wu [28], Human microbiome project [44], Turnbaugh [8], and Yatsunenکو [45].

*“Studies from high impact journals”*

Small differences within the studies – huge differences between studies

# Other possible caveats

**Sinha et al., Genome Biology 2015:** Preliminary results  
”... DNA extraction method was a major source of variation...”

**Standardising microbiome research:** The microbiome quality control project: baseline study design and future directions ...

... e.g. 16S rRNA amplicon analyses versus shotgun metagenomic approaches

**DETOUR  
ENDS**

The wonderful thing about the gut microbiota studies

We will know in great detail about the gut anaerobes

This is extremely useful from a clinical perspective

A couple of examples (including MALDI-TOF)

# *Ruminococcus gnavus*

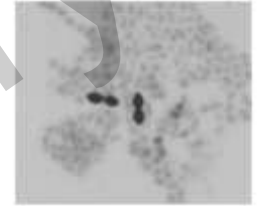


FIG. 2. Gram and phase-contrast images of *Ruminococcus gnavus* (the source of the clinical *Ruminococcus gnavus* strains).

CASE REPORT

## Two Cases of *Ruminococcus gnavus* Bacteremia Associated with Diverticulitis

Sanne G. K. Hansen,<sup>a</sup> Marianne N. Skov,<sup>b</sup> Ulrik S. Justesen<sup>b</sup>

Department of Clinical Microbiology, Lillebaelt Hospital, Vejle, Denmark<sup>a</sup>; Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark<sup>b</sup>

We report two cases of bacteremia with the anaerobic bacterium *Ruminococcus gnavus*. In both cases, the bacteremia was associated with diverticular disease. Preliminary conventional identification suggested peptostreptococci, and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) analysis did not produce scores high enough for species identification. Finally, the bacteria were identified by 16S rRNA gene sequencing.

A gut anaerobe (not a skin anaerobe)  
– the value of MALDI-TOF  
and optimisation (Score 1.723)

In the subsequent Biotyper version 3.1, the database still contained only *R. gnavus*, but one more strain had been entered into the database. Identification of the isolates was repeated with the new database, version 3.1, and *R. gnavus* was suggested, with maximum scores of 2.181 and 2.124.

# *Clostridium celatum*



Journal of  
Clinical Microbiology



CASE REPORT

## Two Serious Cases of Infection with *Clostridium celatum* after 40 Years in Hiding?

Charlotte Nielsen Agergaard, Silje Vermedal Hoegh, Hanne Marie Holt, Ulrik Stenz Justesen

Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark

*Clostridium celatum* [ce.la'tum. L. adj. *celatum* hidden] has been known since 1974, when it was isolated from human feces. In 40 years, no association with human infection has been reported. In this work, we present two serious cases of infection with the anaerobic Gram-positive rod *Clostridium celatum*.

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TABLE 1 Results from identification and antimicrobial susceptibility testing

Parameter	Result(s)	
	Blood isolate 1	Tissue isolate 3
MALDI-TOF MS		
Vitek MS (% match)	<i>C. clostridioforme</i> (99.9)	<i>C. clostridioforme</i> (98.9)
Biotyper (score)	<i>C. disporicum</i> (1.767)	<i>C. disporicum</i> (1.808)
API RapidID 32A (% match)	<i>C. tertium</i> (80.3)	<i>C. clostridioforme</i> (99.3)
Numerical profile	4701004000	4161004000

A gut anaerobe

– the value of MALDI-TOF  
and optimisation

# *Clostridium difficile* infection

Returning to the health and disease and the best example on how to exploit gut anaerobes:

Bugs better than drugs – faecal transplantation

ATTENTION ON: Cammarota et al., Gut 2017 (in press)



**OPEN ACCESS**

European consensus conference on faecal microbiota transplantation in clinical practice

-> mainly focusing on what not to include...

-> however, what to include, is more in the dark ...  
- the case of “synthetic microbiota”

## Rectal bacteriotherapy for recurrent *Clostridium difficile*-associated diarrhoea: results from a case series of 55 patients in Denmark 2000–2012

64% had no recurrence within 30 days of bacteriotherapy.

**TABLE 1.** Composition of bacterial culture used for rectal bacteriotherapy treatment in 55 patients with recurrent *Clostridium difficile*, Denmark 2000–2012

1.	<i>Bacteroides ovatus</i>	MT IS-la
2.	<i>Escherichia coli</i> 1108	MT IS-lb
3.	<i>E. coli</i> 1109	MT IS-le
4.	<i>Streptococcus faecalis</i>	MT IS-lf
5.	<i>Lactobacillus</i> sp	MT IS-le
6.	<i>Bacteroides thetaiotaomicron</i>	MT IS-lf
7.	<i>Bacteroides vulgatus</i>	MT IS-lg
8.	<i>Clostridium bifermentans</i>	MT IS-lh
9.	<i>Clostridium innocuum</i>	MT IS-2a
10.	<i>Clostridium ramosum</i>	MT IS-2b
11.	<i>Lactobacillus rhamnosus</i>	19070-2
12.	<i>Lactobacillus acidophilus</i>	18911-2

Bacterial concentration per species was approximately  $10^{10}$ /mL.

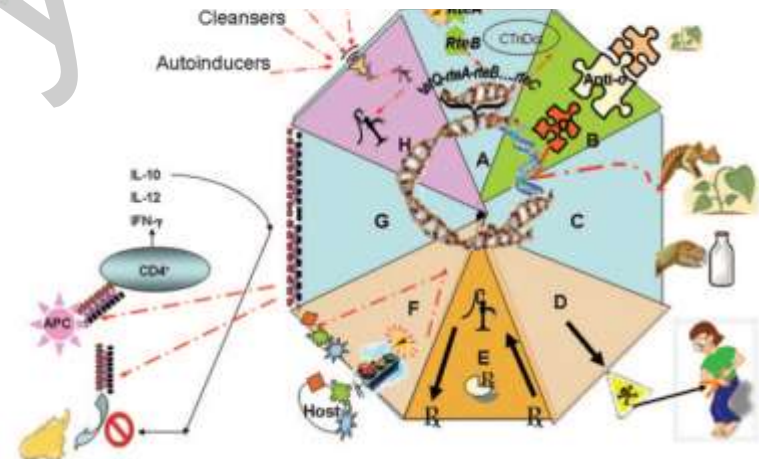


## *Bacteroides*: the Good, the Bad, and the Nitty-Gritty

Hannah M. Wexler\*

*Wadsworth Anaerobe Laboratory, Greater Los Angeles VA Healthcare Systems, and Department of Medicine,  
University of California, Los Angeles, California*

The aspects of these organisms that will be covered will include their role as commensal organisms (The Good); their involvement in human disease (The Bad); and information about their physiology, metabolism, and resistance mechanisms as well as a brief overview of clinical characteristics (The Nitty-Gritty).



# The *Bacteroides fragilis* group

## Good or bad (the double-edged sword)?

- From the metabolic point of view?
  - they are apparently good (*Firmicutes/Bacteroidetes* ratio)
- From blood cultures (most frequent anaerobe)?
  - not so good – high mortality rates



# The *Bacteroides fragilis* group

## Good or bad (the double-edged sword) - continued?

- Increasing resistance - good or bad?
  - good if your gut is in perfect condition and you are going to receive antibiotics and don't want to be obese???
  - bad if you have colon cancer and/or are going to have an operation

*“Our good normal flora fighting back?”*



Antimicrobial resistance in the *Bacteroides fragilis* group in faecal microbiota from healthy Danish children <sup>□</sup>

Thomas Vognbjerg Sydenham <sup>4,\*</sup>, Betina Hebbelstrup Jensen <sup>1</sup>, Andreas Munk Petersen <sup>3†</sup>, Karen Angeliki Kroghfelt <sup>1</sup>, Ulrik Stenz Justesen <sup>1</sup>

<sup>4</sup> Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark

<sup>1</sup> Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark

<sup>3</sup> Department of Clinical Microbiology, Hvidovre University Hospital, Copenhagen, Denmark

<sup>2</sup> Department of Gastroenterology, Hvidovre University Hospital, Copenhagen, Denmark

# The central nervous system and the gut microbiome

Lots of associations..., e.g. the gut microbiome in the development of autism and the role of microbial dysbiosis

**Good:** e.g. *Lactobacillus reuteri*

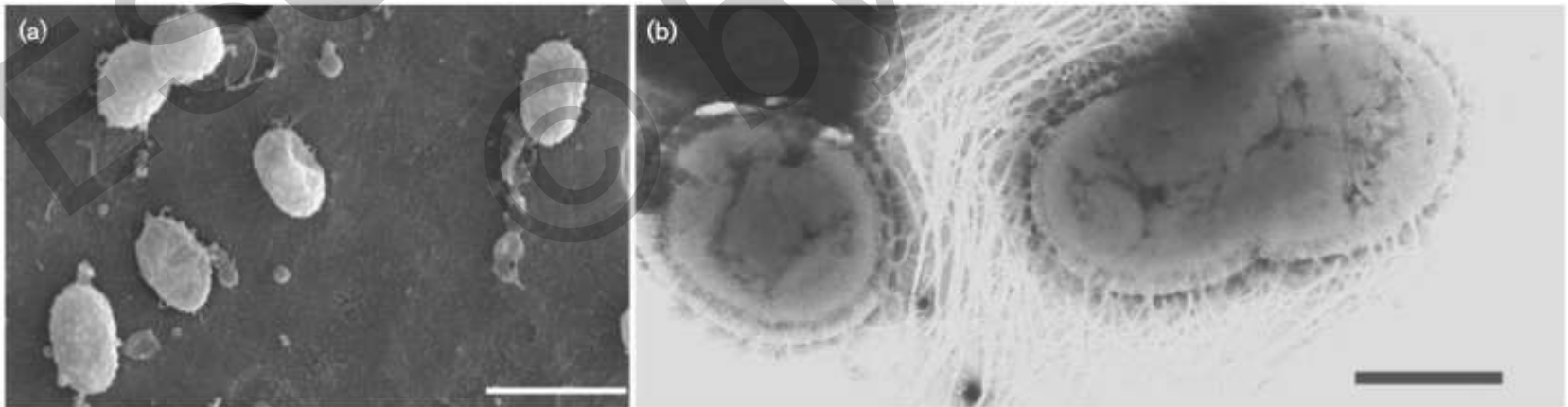
**Bad:** e.g. *Desulfovibrio*

*"Enterococcus hirae is protective against multiple sclerosis"*



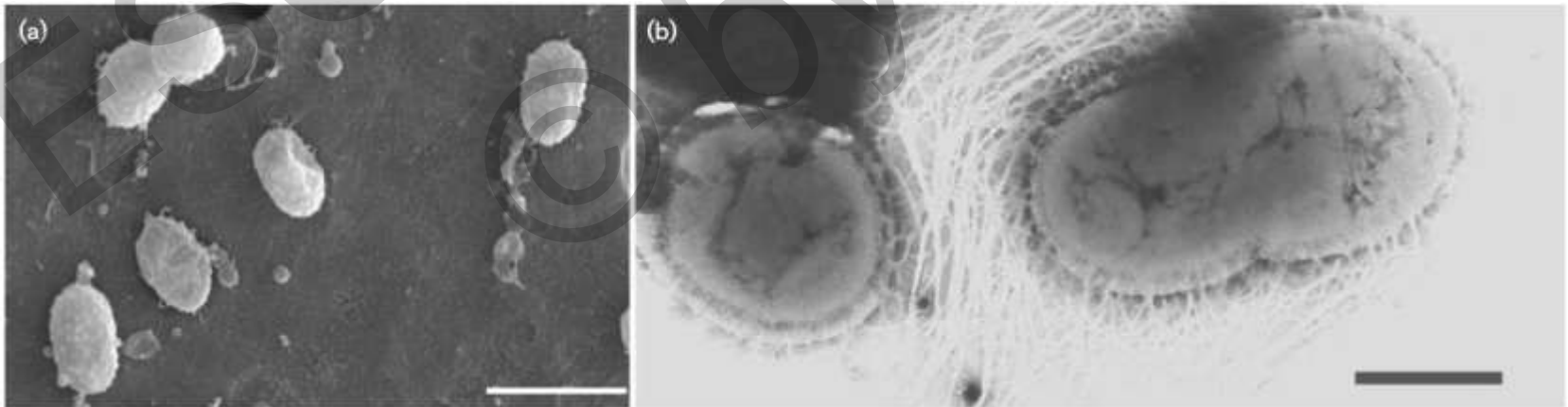
# *Akkermansia muciniphila*

- From Antoon Akkermans, a Dutch microbiologist (Derrien et al., IJSEM 2004)
- Gram-negative, strictly **ANAEROBIC**, non-motile, non-spore-forming rod
- Mucin degrading bacteria – good or bad – apparently good???



# *Akkermansia muciniphila*

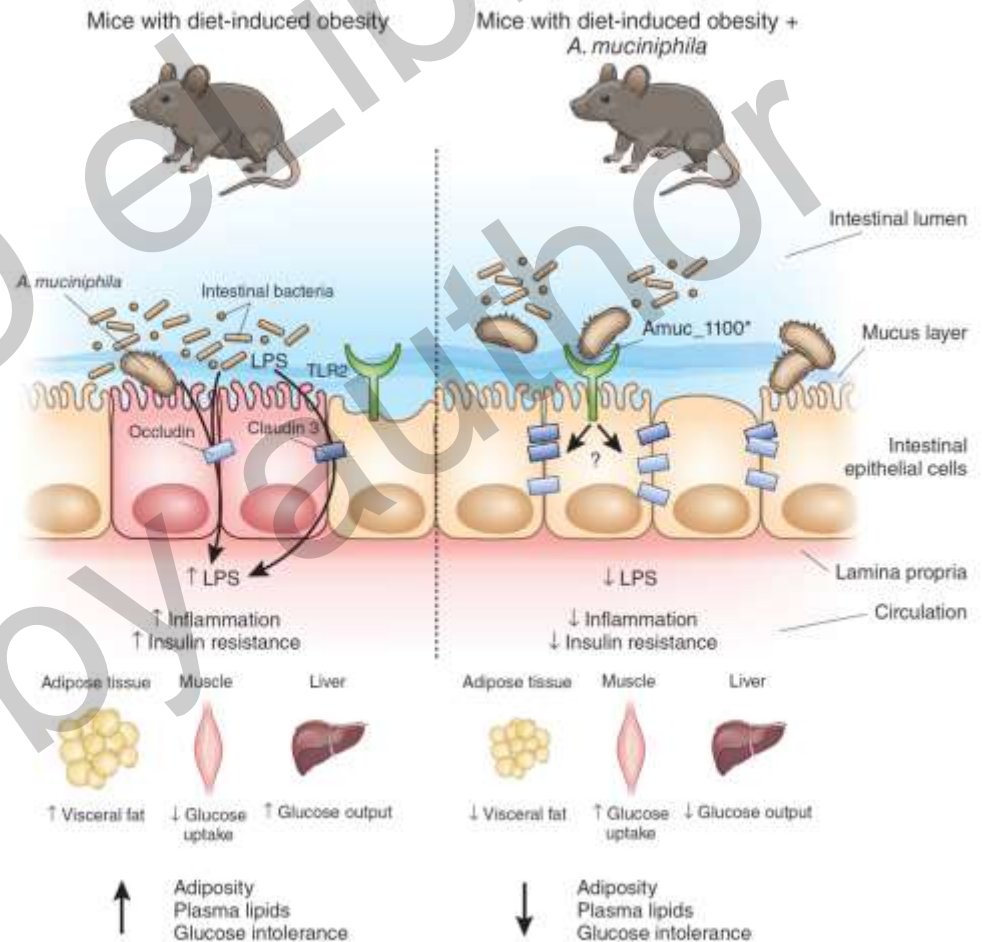
- Named as the “*Weight loss bacterium*”
- It was “bacterium of the month – august 2016” on [www.ubiomeblog.com](http://www.ubiomeblog.com)
- From association, over causal relationship to treatment



# Akkermansia muciniphila

Anhê and Marette, Nat Med 2017:  
News and views on paper by Plovier  
et al., Nat Med 2017:

- Proof-of-concept in mice with diet-induced obesity:
- Administration of pasteurized *A. muciniphila* or the thermostable outer-membrane protein (Amuc\_1100\*) of *A. muciniphila* reduced the fat mass.
- Not just association but causal relationship



# *Akkermansia muciniphila*

- Furthermore treatment of overweight humans with live or pasteurized *A. muciniphila* were safe and well tolerated ...and then the paper ends with:
- *“These results pave the way for future human studies investigating A. muciniphila as a therapeutic tool in the management of the metabolic syndrome.”*

**We are waiting!!!**





# *Akkermansia muciniphila*

- However, apparently colleagues from Marseille have almost spoiled the fun:

## Accepted Manuscript

First isolation of *Akkermansia muciniphila* in a blood culture sample

Grégory Dubourg, Florent Cornu, Sophie Edouard, Alexandre Battaini, Michel Tsimaratos, Didier Raoult



- Dubourg et al., CMI 2017 in press
- Diagnosis with MALDI-TOF

## But, but, but ...

- Every bug with just a little self-respect has been the cause of endocarditis and have a least one case report on PubMed
- Do you need to consider endocarditis each time you find these bugs in a blood culture?
- However, each time you find a gut anaerobe in a blood culture you need to consider a problem within the gut, although the patient might not be very sick from bacteremia.



# In gut we trust

- We have learnt so much from the studies on the gut microbiota – especially which anaerobes belong to the gut
- This makes it much easier to interpret the results from MALDI-TOF in a disease situation
- Disease might eventually promote health (*S. bovis* group analogue)

