

# Cultivating the uncultured

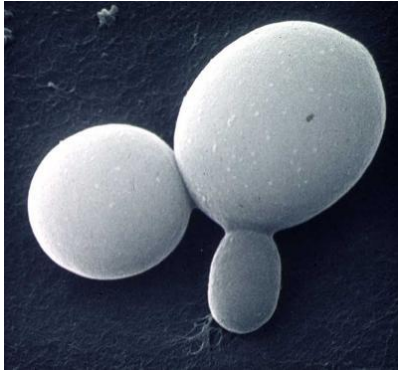
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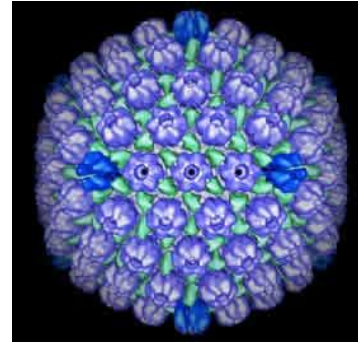
# Outline

- The human oral microbiome
- Uncultured oral bacteria
- Culturing uncultured bacteria

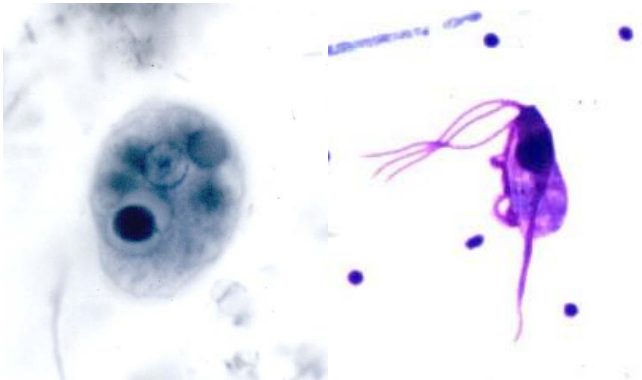
# The oral microbiome



Fungi



Viruses



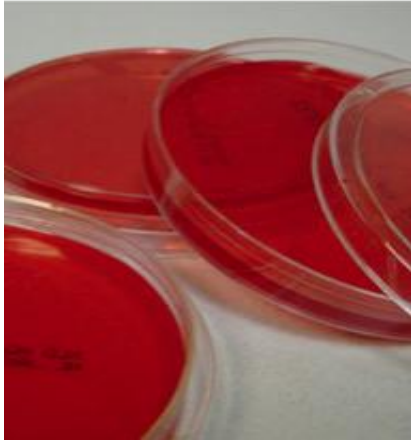
Protozoa



## Bacteria and Archaea

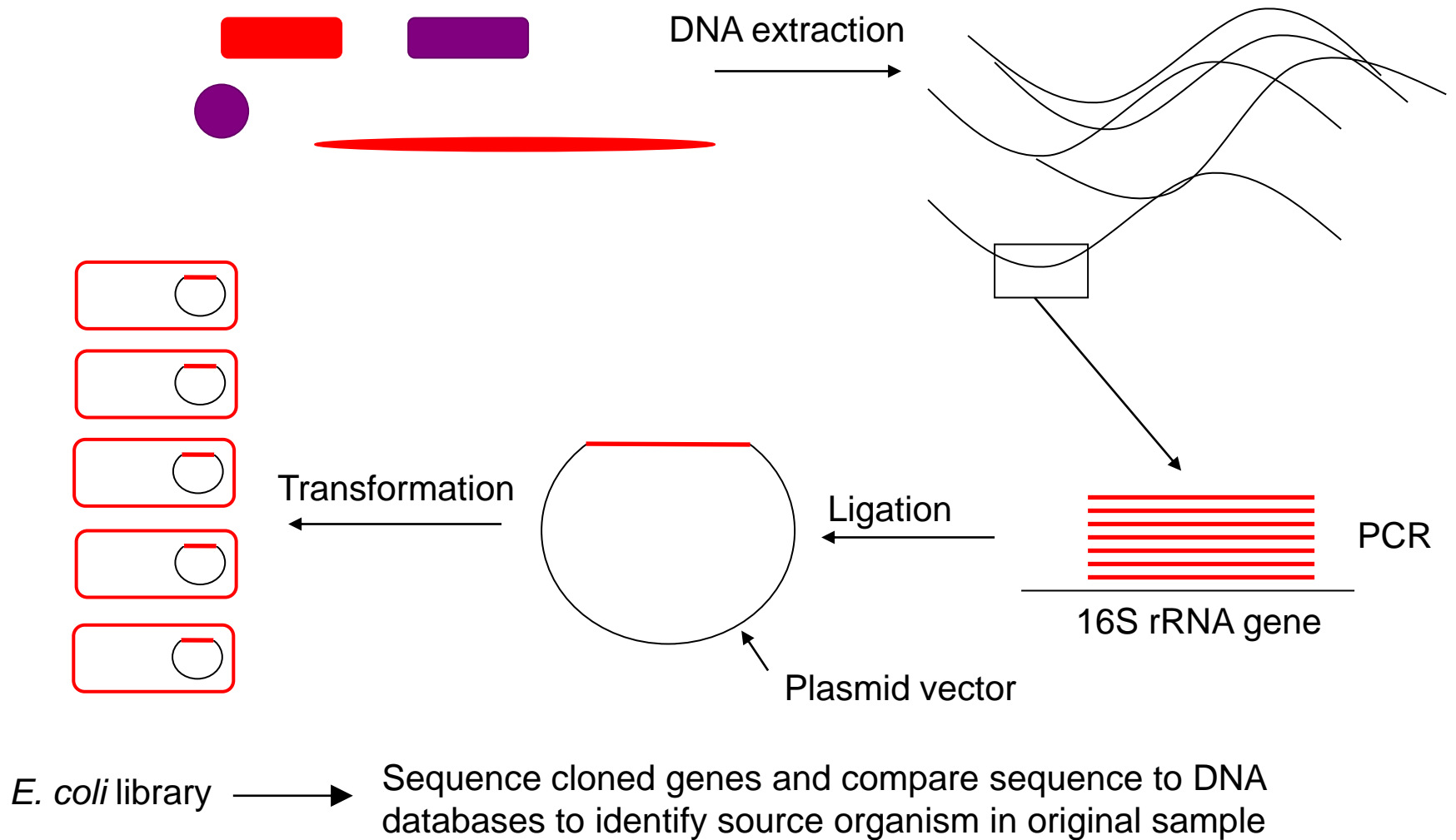
- Saliva -  $10^8$  / ml
- All oral surfaces colonised by biofilm
- Important role in tooth decay and gum disease

# Cultural analysis of oral bacteria



- Oral bacteria typically fastidious and slow-growing – require complex media and long incubation times
- Many are strict anaerobes requiring care in sample collection, transport and incubation
- Comprehensive cultural analysis of samples difficult – only possible to analyse small number
- Around half of oral bacteria uncultivable

# 16S rRNA community profiling



# Next generation sequencing

- High throughput – e.g. Illumina MiSeq generates c 10M sequences per run
- Direct sequencing of amplicons – no cloning step
- For oral bacteria, V1-V2 is most informative
- Easy and quick to do with standard pipelines for analysis – explosion in publications



## Taxon Description

- [Taxon Table](#)
- [Taxonomic Hierarchy](#)
- [Taxonomic Level](#)

## Identify 16S rRNA Sequence

- [BLAST Sequence\(s\)](#)
- [16S rDNA RefSeq Download](#)

## Genomes

- [Taxa with Annotated Genomes](#)
- [All Oral Genomes](#)
- [All Genomes](#)

## Tools &amp; Download

- [HOMD Genome Viewer](#)
- [HOMD JBrowse Viewer »](#)
- [View Dynamic Annotation](#)
- [BLAST Against HOMD Genomes »](#)
- [Dynamic Genome BLAST](#)
- [Download HOMD Data](#)
- [HOMD Posters](#)
- [Oralgen »](#)

## HOMD Information

- [How to cite HOMD](#)
- [Project Description](#)
- [Strains and DNA Availability](#)
- [Team](#)
- [Contact Us](#)
- [Mailing List](#)
- [Help & Guides »](#)
- [Usage Statistics](#)
- [Site Map](#)

## Welcome to HOMD

The goal of creating the Human Oral Microbiome Database (HOMD) is to provide the scientific community with comprehensive information on the approximately 700 prokaryote species that are present in the human oral cavity. Approximately 54% are officially named, 14% unnamed (but cultivated) and 32% are known only as uncultivated phylotypes. The HOMD presents a provisional naming scheme for the currently unnamed species so that strain, clone, and probe data from any laboratory can be directly linked to a stably named reference scheme. The HOMD links sequence data with phenotypic, phylogenetic, clinical, and bibliographic information. Genome sequences for oral bacteria determined as part of this project, the Human Microbiome Project, and other sequencing projects are being added to the HOMD as they become available. Genomes for 400 oral taxa (58% of taxa on HOMD) are currently available on HOMD. The HOMD site offers easy to use tools for viewing all publically available oral bacterial genomes. Welcome!

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## Meta-Database Search

## Announcement

2015-12-08 11:49:40

Publication: Robust species taxonomy assignment algorithm for 16S rRNA NGS reads: application to oral carcinoma samples [\[more\]](#)

2014-10-07 15:02:46

Important Notice Regarding Tannerella forsythia (strain 92A2) [\[more\]](#)

2014-03-21 14:55:41

The Los Alamos ORALGEN Database Archive [\[more\]](#)



## Database Update

2016-02-26 23:02

Genome Annotation Update - Staphylococcus warneri L37603 [\[more\]](#)

2016-02-26 17:02

Genome Annotation Update - Streptococcus vestibularis F0396 [\[more\]](#)

2016-02-26 13:02

Genome Annotation Update - Selenomonas sputigena ATCC 35185 [\[more\]](#)

2016-02-26 09:02

Genome Annotation Update - Streptococcus sobrinus TCI-107 [\[more\]](#)





## Human Oral Microbial Taxa with Annotated Genomes

[Previous page](#)

Total: 151 taxa

Oral Taxon ID (HOT)	Genus	Species	Status	Flag	Taxon Link	Genome Link
389	<i>Abiotrophia</i>	<i>defectiva</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
554	<i>Acinetobacter</i>	<i>baumannii</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
176	<i>Actinomyces</i>	<i>naeslundii</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
701	<i>Actinomyces</i>	<i>odontolyticus</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
848	<i>Actinomyces</i>	<i>sp. oral taxon 848</i>	Unnamed		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
531	<i>Aggregatibacter</i>	<i>actinomycetemcomitans</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
485	<i>Agrobacterium</i>	<i>tumefaciens</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
738	<i>Anaerococcus</i>	<i>prevotii</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
788	<i>Anaerococcus</i>	<i>tetradus [NV]</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
723	<i>Atopobium</i>	<i>parvulum</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
750	<i>Atopobium</i>	<i>rimae</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
814	<i>Atopobium</i>	<i>vaginae</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
824	<i>Bacillus</i>	<i>anthracis</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
045	<i>Bacillus</i>	<i>clausii</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
468	<i>Bacillus</i>	<i>subtilis</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>
588	<i>Bifidobacterium</i>	<i>dentium</i>	Named		<a href="#">Taxon Description</a>	<a href="#">View Genome</a>



# The human oral microbiome

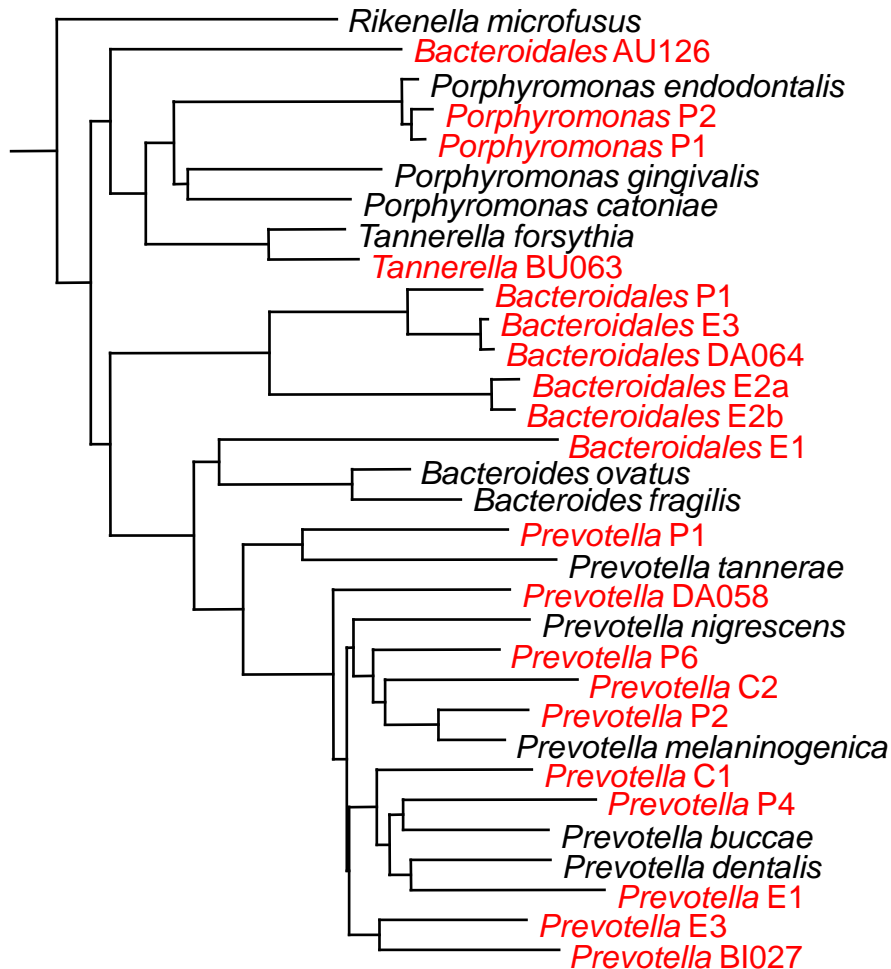
(Dewhirst et al., J. Bacteriol., 2010)

- 687 bacterial species-level taxa
- 6 phyla – *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Spirochaetes* and *Fusobacteria* account for 96 % of species
- 116 cultured but un-named species
- 69 % of species cultured

# Sequences vs cells

- DNA sequence information is valuable resource:
  - Can be used as biomarkers
  - Enables predictions of function
- But, for:
  - Experimental determination of function
  - Use in model systems
  - Potential use as probiotics
- Culture and description and naming of bacteria is required

# Uncultivated taxa - *Bacteroidetes*



- Some belong to existing well-characterised genera
- Others constitute deep-branching lineages with no cultivable representatives

# Reasons for lack of cultivation

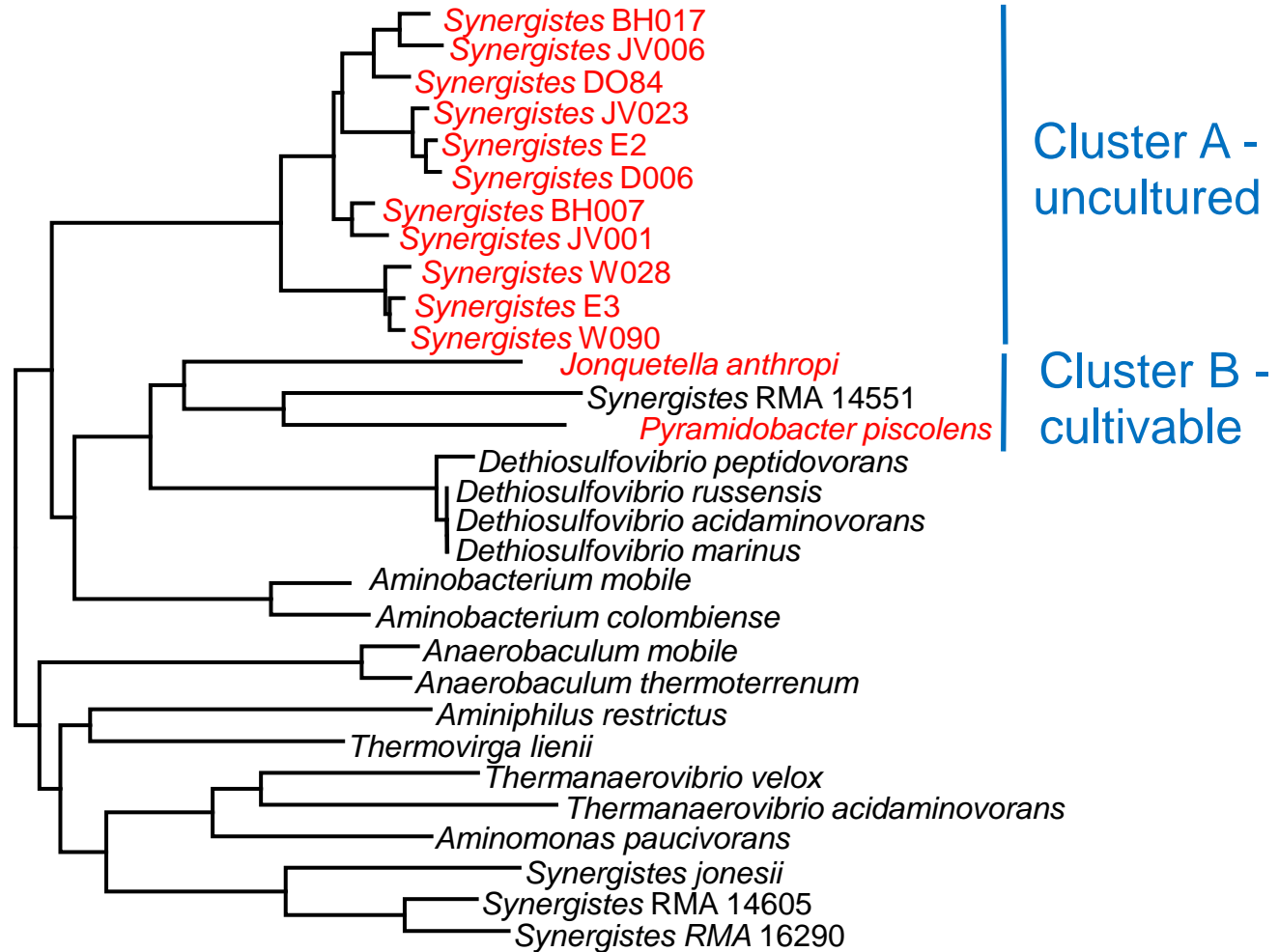
- Undersampling – culture much more labour-intensive than culture-independent studies
  - High throughput culture reveals many “uncultured” bacteria; e.g. Browne et al. Nature 2016; 533: 543-6 - substantial proportion of intestinal bacteria cultured
- Dependence on other bacteria in the community
  - nutritional / signaling
  - epibionts and / or intracellular, e.g. TM7 (He et al. PNAS 2015;112:244-9)

# **“Microbioal Dark matter”**

- Hug et al. A new view of the tree of life. Nature Microbiol 2016. 48
- Examination of environmental metagenomic sequence data revealed novel branch of phylogenetic tree largely unseen in 16S rRNA gene community profiling surveys - “Candidate Phyla Radiation”
- Mis-matches with currently used primers and inserts in 16S rRNA operons
- Some representatives of the CPR have small genomes and may be epibionts or parasitic on other bacteria

# The phylum *Synergistetes*

(Jumas-Bilak et al. 2009)



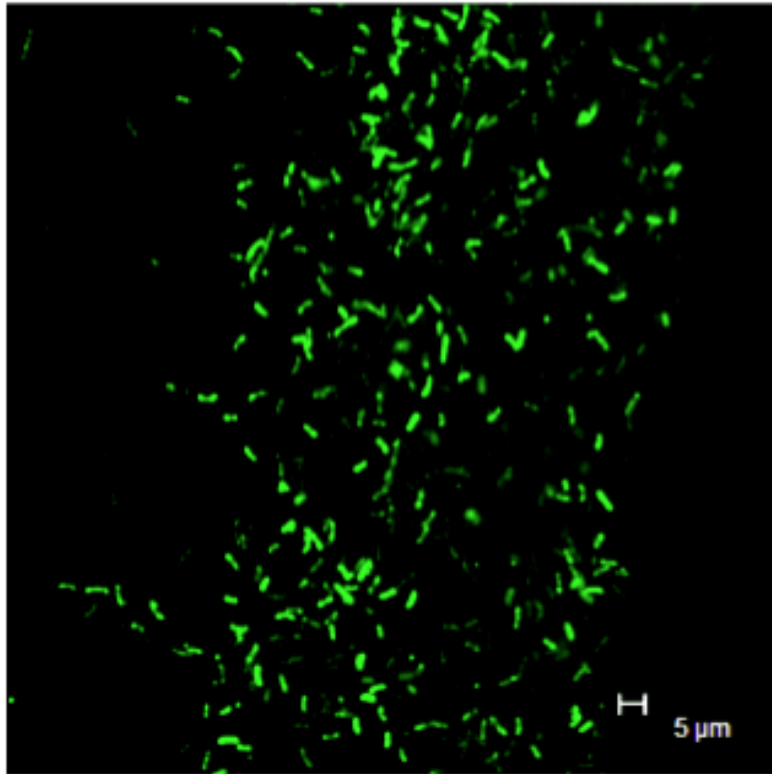
# Culturing an uncultivated *Synergistetes*

(Vartoukian et al. Environ Microbiol 2010;12:916-28)

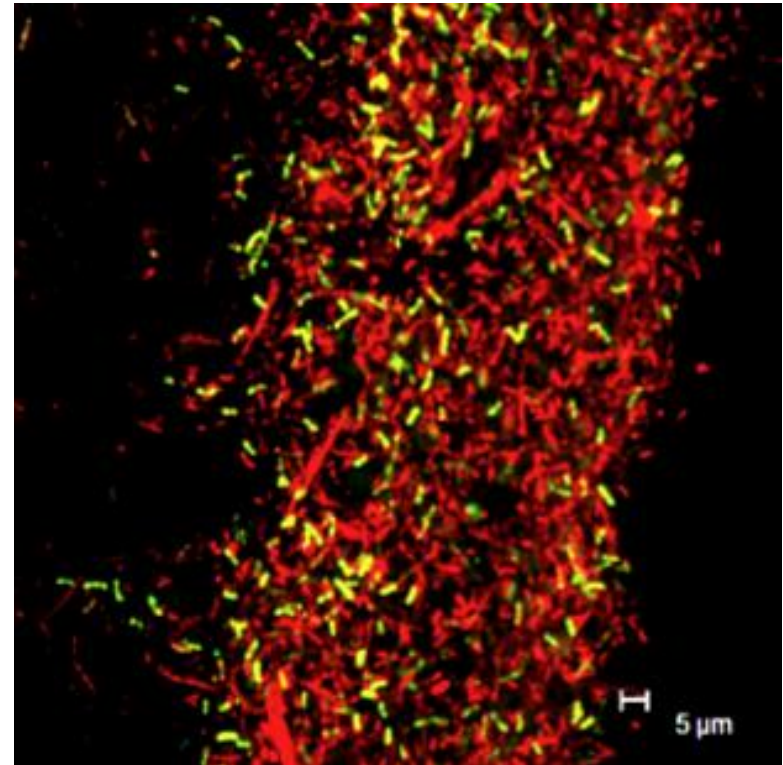
- Hypothesis: some uncultivated oral bacteria require presence of other bacteria and therefore could be grown in mixed culture *in vitro*
- Aim of study: to cultivate representative of uncultivated *Synergistetes* to obtain a pure culture

# Plaque from periodontal pocket

(Vartoukian et al. Appl Env Microbiol 2009;75:3777-86)



*Synergistetes* Cluster A

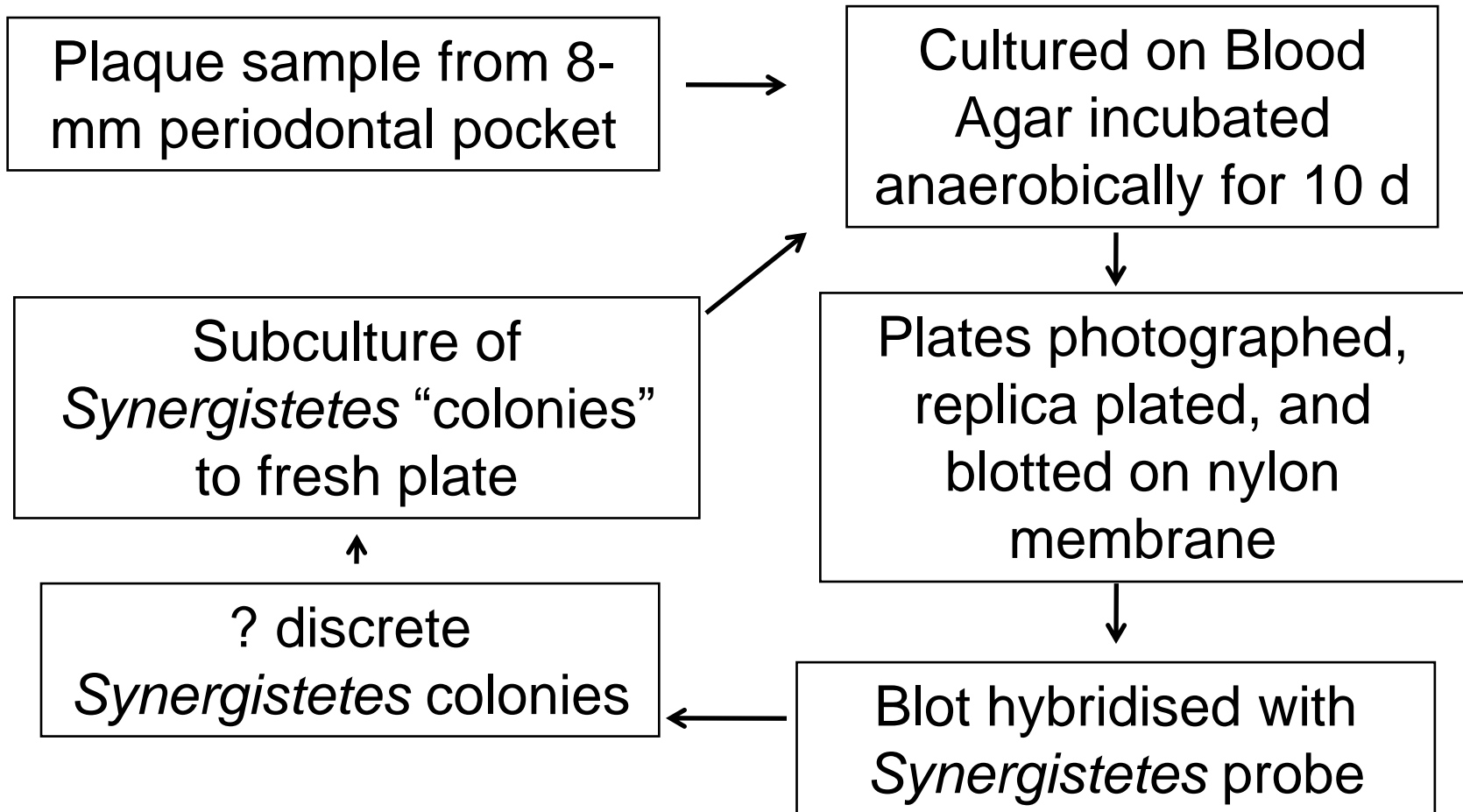


Total bacteria (in red) with  
*Synergistetes* Cluster A

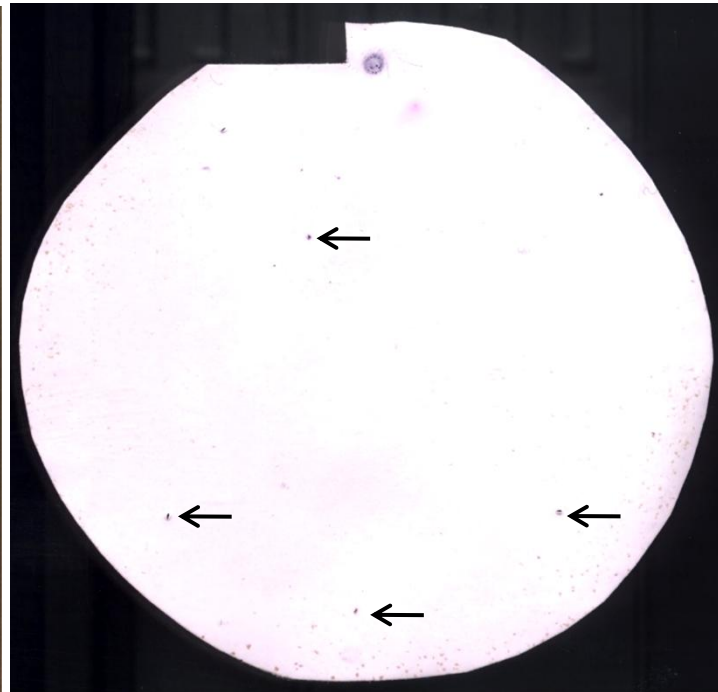
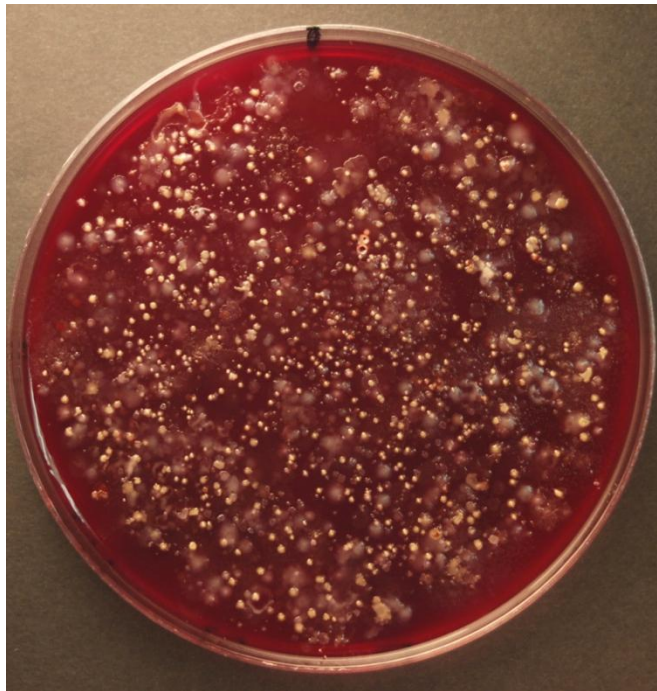


# Method

(Vartoukian et al. Environ Microbiol 2010;12:916-28)



# *Synergistetes* colony hybridisation of mixed primary culture

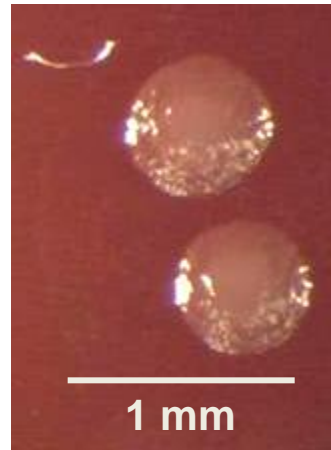
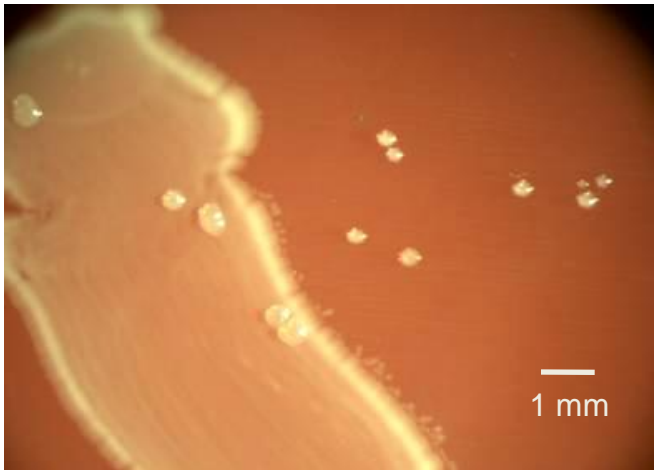


- Detection of scanty growth of *Synergistetes*
- Replica plate harvested in region of hybridisation and used to inoculate fresh plate

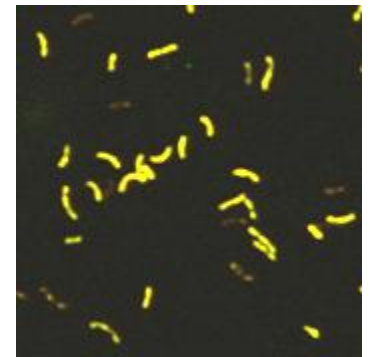
# Composition of enriched community after 8 passages

- Culture:
  - *Parvimonas micra*
  - *Campylobacter rectus*
  - *Anaeroglobus geminatus*
  - *Tannerella forsythia*
- Clone library
  - As above plus:
  - *Synergistetes* phylotype W090

# Passage 12, with *P. micra* streak



Clone library –  
*Synergistetes* W090 only



# Species naming and genome sequence

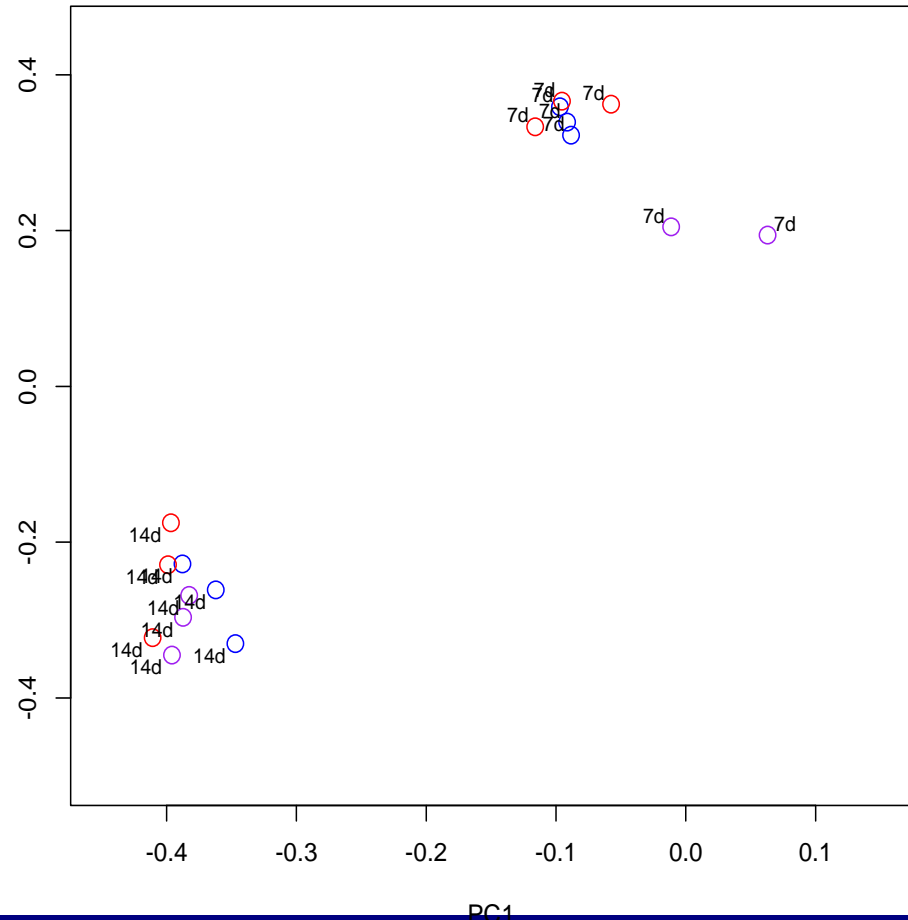
- Organism described and named *Fretibacterium fastidiosum* (Vartoukian et al. 2012)
- DNA extraction – small amount obtained
- Whole Genome Amplification (GenomiPhi)
- Sequenced at Sanger Institute
- Accession no. FP929056

# Development and pyrosequencing analysis of an in-vitro oral biofilm model

(Kistler et al. BMC Microbiol 2015;15:24.)

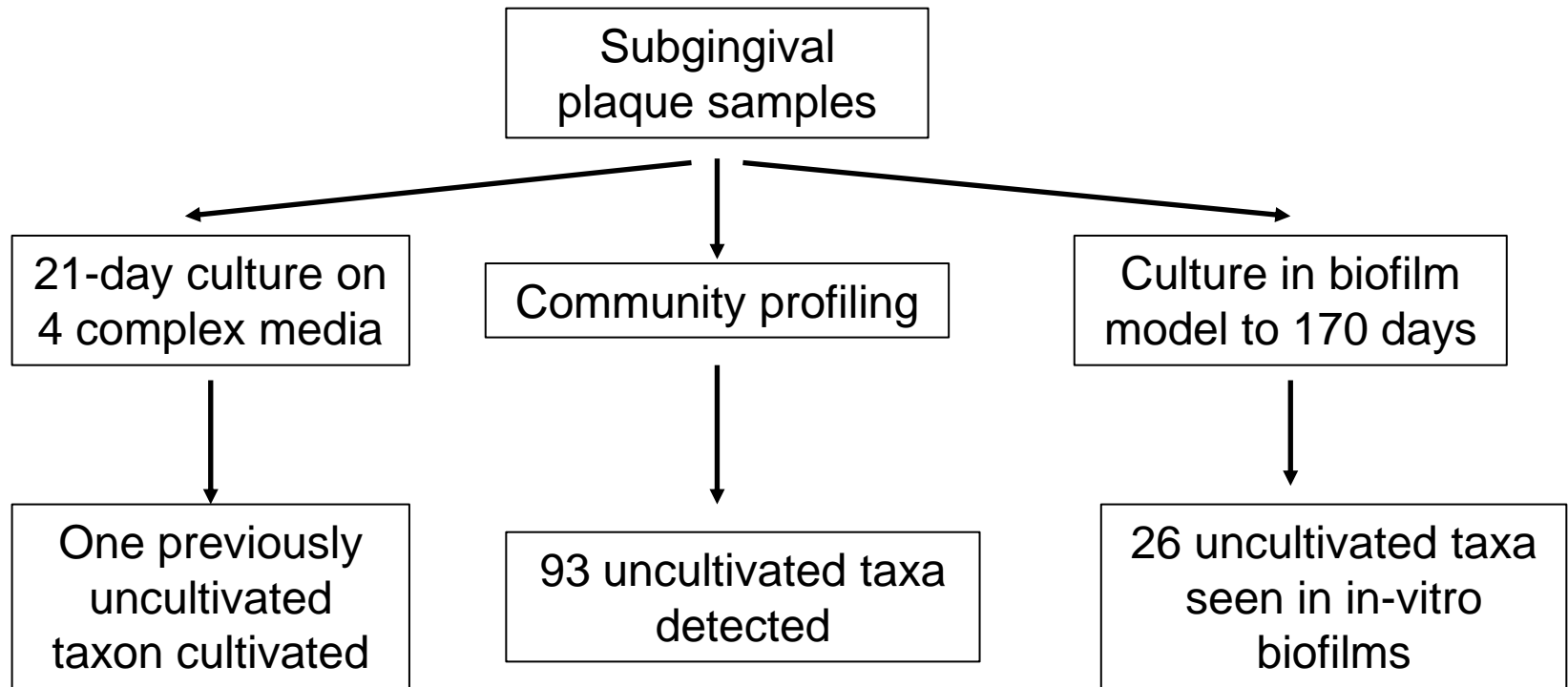


- Hydroxyapatite-coated pegs
- Inoculated with saliva
- Media changed twice weekly
- Complex biofilm develops, with >250 OTUs / peg



# In-vitro culture of previously uncultured oral bacterial phylotypes

(Thompson et al. Appl. Environ. Microbiol. 2015;81:8307-8314)



# Culture of uncultivated oral taxa

Thompson et al. Appl Environ Microbiol. 2015;81:8307-14.

Combination of in-vitro biofilm as source and targeted colony hybridisation



*Lachnospiraceae* HOT 500

*Veillonella dispar* / *parvula*

*Parvimonas micra*

Vartoukian et al. PLoS One.

2016;11:e0146926.

Isolated in co-culture with helper strains:

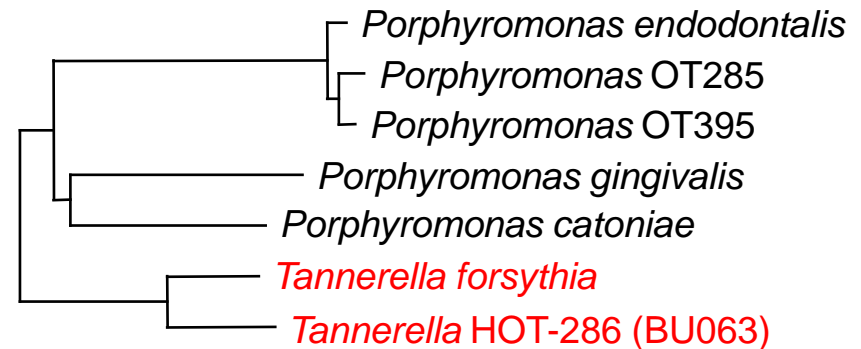
- *Anaerolineae* HOT-439, the first oral taxon from the *Chloroflexi* phylum
- *Bacteroidetes* HOT-365
- *Peptostreptococcaceae* HOT-091



# *Tannerella forsythia* and *Tannerella* BU063

(Leys et al. J Clin Microbiol 2002;40:821-5)

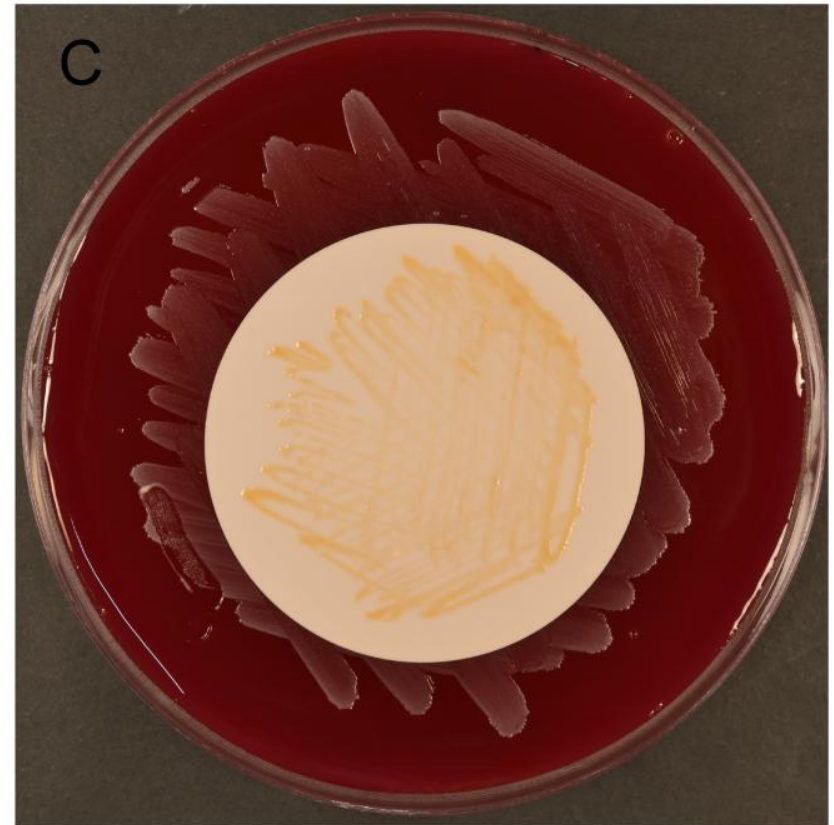
- *T. forsythia* strongly associated with periodontitis
- Uncultivated *Tannerella* phylotype BU063 (HOT-286) strongly associated with health



# First Cultivation of Health-Associated *Tannerella* sp. HOT-286 (BU063)

(Vartoukian et al. J Dent Res 2016;95:1308-1313)

- Cultivated from subgingival plaque
- Stimulated by *Propionibacterium acnes*
- Grew on membrane placed on *P. acnes* culture



# Summary

- Culture is important!
- Oral bacteria have evolved as a community and many species are dependent on community lifestyle
- Microbial dark matter needs to be explored - extent of representation among human microbiome not yet clear
- New methods of co-culture are proving successful (but slow!) in cultivating previously uncultured oral bacteria

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