The oral microbiome: we know who’s there, but what are they doing?

R. J. Palmer Jr., Ph.D.
NIDCR/NIH
Antonie van Leeuwenhoek
1632 - 1723

"a little white matter, which is as thick as if 'twere batter."
The Great Plate-count Anomaly (1970s)

- seawater
- petri dish
- filter disc

allow bacterial colonies to develop

= bacteria per unit volume

examine in microscope

= bacteria per unit volume

# bacteria counted in microscope is >> # of colonies seen on plates

ca. 1% of bacteria had been cultivated
CARL WOESE
1929 – 2012

MacArthur Fellow
Crafoord prize
Leeuwenhoek medal
US Nat’l Academy Sci
Royal Society
conserved variable

ribosome is a “molecular clock”
1) universal
2) conserved and variable regions
3) small subunit (16S, 18S) ideal size for sequencing
Phylogenetic structure of the prokaryotic domain: The primary kingdoms

(archaeabacteria/eubacteria/urkaryote/16S ribosomal RNA/molecular phylogeny)

CARL R. WOESE AND GEORGE E. FOX*

Department of Genetics and Development, University of Illinois, Urbana, Illinois 61801

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Table 1. Association coefficients ($S_{AB}$) between representative members of the three primary kingdoms

<table>
<thead>
<tr>
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<th>1</th>
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<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
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</thead>
<tbody>
<tr>
<td>1. Saccharomyces cerevisiae, 18S</td>
<td>---</td>
<td>0.29</td>
<td>0.33</td>
<td>0.05</td>
<td>0.06</td>
<td>0.08</td>
<td>0.09</td>
<td>0.11</td>
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<td>0.11</td>
<td>0.11</td>
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<tr>
<td>2. Lema minor, 18S</td>
<td>0.29</td>
<td>---</td>
<td>0.36</td>
<td>0.10</td>
<td>0.05</td>
<td>0.06</td>
<td>0.10</td>
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<td>0.10</td>
<td>0.10</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>3. L cell, 18S</td>
<td>0.33</td>
<td>0.36</td>
<td>---</td>
<td>0.06</td>
<td>0.06</td>
<td>0.07</td>
<td>0.07</td>
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<td>0.10</td>
<td>0.10</td>
<td>0.09</td>
<td>0.07</td>
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<tr>
<td>4. Escherichia coli</td>
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<td>0.10</td>
<td>0.06</td>
<td>---</td>
<td>0.24</td>
<td>0.25</td>
<td>0.28</td>
<td>0.26</td>
<td>0.21</td>
<td>0.11</td>
<td>0.12</td>
<td>0.07</td>
<td>0.12</td>
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<tr>
<td>5. Chlorobium vibrioforme</td>
<td>0.06</td>
<td>0.05</td>
<td>0.06</td>
<td>0.24</td>
<td>---</td>
<td>0.22</td>
<td>0.22</td>
<td>0.20</td>
<td>0.19</td>
<td>0.06</td>
<td>0.07</td>
<td>0.06</td>
<td>0.09</td>
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<tr>
<td>6. Bacillus firmus</td>
<td>0.08</td>
<td>0.06</td>
<td>0.07</td>
<td>0.25</td>
<td>0.22</td>
<td>---</td>
<td>0.34</td>
<td>0.26</td>
<td>0.20</td>
<td>0.11</td>
<td>0.13</td>
<td>0.06</td>
<td>0.12</td>
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<tr>
<td>7. Corynebacterium diphtheriae</td>
<td>0.09</td>
<td>0.10</td>
<td>0.07</td>
<td>0.28</td>
<td>0.22</td>
<td>0.34</td>
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<td>0.23</td>
<td>0.21</td>
<td>0.12</td>
<td>0.12</td>
<td>0.09</td>
<td>0.10</td>
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<tr>
<td>8. Aphanocapsa 6714</td>
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<td>0.09</td>
<td>0.26</td>
<td>0.20</td>
<td>0.28</td>
<td>0.23</td>
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<td>0.31</td>
<td>0.11</td>
<td>0.11</td>
<td>0.10</td>
<td>0.10</td>
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<td>10. Methanobacterium thermoautotrophicum</td>
<td>0.11</td>
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<td>0.10</td>
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<td>0.06</td>
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<td>0.12</td>
<td>0.11</td>
<td>0.14</td>
<td>---</td>
<td>0.51</td>
<td>0.25</td>
<td>0.30</td>
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<td>11. M. ruminantium strain M-1</td>
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<td>0.10</td>
<td>0.12</td>
<td>0.07</td>
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<td>0.12</td>
<td>0.11</td>
<td>0.12</td>
<td>0.51</td>
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<td>0.25</td>
<td>0.24</td>
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<tr>
<td>12. Methanobacterium sp., Cariacoisolate JR-1</td>
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<td>0.13</td>
<td>0.09</td>
<td>0.07</td>
<td>0.06</td>
<td>0.06</td>
<td>0.09</td>
<td>0.10</td>
<td>0.10</td>
<td>0.25</td>
<td>0.25</td>
<td>---</td>
<td>0.32</td>
</tr>
<tr>
<td>13. Methanosarcina Barkeri</td>
<td>0.08</td>
<td>0.07</td>
<td>0.07</td>
<td>0.12</td>
<td>0.09</td>
<td>0.12</td>
<td>0.10</td>
<td>0.10</td>
<td>0.12</td>
<td>0.30</td>
<td>0.24</td>
<td>0.32</td>
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</table>

The 16S (18S) ribosomal RNA from the organisms (organelles) listed were digested with T1 RNase and the resulting digests were subjected to two-dimensional electrophoretic separation to produce an oligonucleotide fingerprint. The individual oligonucleotides on each fingerprint were then sequenced by established procedures (13, 14) to produce an oligonucleotide catalog characteristic of the given organism (3, 4, 13–17, 22, 23; unpublished data). Comparisons of all possible pairs of such catalogs defines a set of association coefficients ($S_{AB}$) given by: $S_{AB} = 2N_{AB}/(N_A + N_B)$, in which $N_A$, $N_B$, and $N_{AB}$ are the total numbers of nucleotides in sequences of hexamers or larger in the catalog for organism A, in that for organism B, and in the intersection of the two catalogs, respectively (13, 23).
The Great Plate-count Anomaly is solved by RNA-based taxonomy

“Unculture-able” bacteria can be detected and classified, but what does this mean to bacterial taxonomy?

valid bacteriological species
  pure culture
  physiology
  sequence data

molecular species
  defined by sequence data
  SLOTU (Species Level Operational Taxonomic Unit)
  OTU
  taxon

all valid bacteriological species are molecular species but NOT vice versa – cultivated organism required
The microbiome of healthy skin is well described by cultivation.

182 OTUs
85% are cultivated
15% are yet to be cultured

Gao et al. (2007)
The microbiome of the healthy gut is not well described by cultivation.

395 OTUs + 1 archeal OTU
80% yet-to-be cultured

stool is not mucosa

Eckburg et al. (2005)
**Tooth surface**
52 OTUs
44% yet-to-be cultured

**Firmicutes - Bacilli**

**Subgingival plaque**
347 OTUs
52% yet-to-be cultured

**Firmicutes - other**

**Entire oral cavity**
ca. 700 OTUs
ca. 60% yet-to-be cultured

**Actinobacteria**

**Synergistes**

**Spirochaetes**

**Fusobacteria**

**Proteobacteria**

**Bacteroidetes**

subgingival plaque

Aas et al. (2005)
Paster et al. (2006)
Flora of diseased periodontal pockets differs from that of healthy pockets

29 periodontally healthy subjects

29 subjects with chronic periodontitis
shallow pockets (“healthy” sites)
deep pockets (“diseased” sites)

direct sequencing of 16s PCR amplicons
Proportions of species in diseased sites differ from those in healthy sites

Griffen et al. 2012
Individuals are ecosystems

Periodontitis-associated species:
- Filifactor alocis, p=0.000036
- Porphyromonas gingivalis, p=0.00052
- Treponema denticola, p=0.000021
- Treponema vincentii/medium, p=0.000075
- Leptotrichia o.t. 210, p=0.000076
- Treponema o.t. 230, p=0.00054

Health-associated species:
- S. mitis group, p=0.0006
- Streptococcus sanguinis, p=0.00026
- Moraxella osloensis, p=0.00037
- Acinetobacter junii, p=0.031
- Granulicatella adiacens, p=0.0011
- Acinetobacter sp. RUH1139, p=0.0046
- S. intermedius group, p=0.03
- Achromobacter wotuwensis, p=0.0096
- A. viscosus group, p=0.00011

- Bacteroides fragilis, p=0.0012
- Lautropia AP009, p=0.006
- Gemella morbillorum, p=0.049

Griffen et al. 2012
Coaggregation: a driver of spatiotemporal community assembly?

Coaggregation is an in vitro assay of cell-cell recognition.

Kolenbrander et al. 2002

add sugar or protease
How can one assess the relevance of coaggregation to biofilms in vivo?

Microscopy provides spatio–temporal data on developing biofilms.
Cell-cell recognition *in vivo*

**Adhesin-Receptor Interactions**

- *S. oralis* serotype 1 RPS
- *S. gordonii* whole cell
- *A. naeslundii*
- *T2 fimbriae*
ALL BACTERIA
STREPTOCOCCAL
RECEPTOR POLYSACCHARIDE
ADHESIN-BEARING
STREPTOCOCCUS

4 hrs

A

10 μm

B

8 hrs

C

Nyvad 1987

D

Palmer 2003
12 hrs

ALL BACTERIA
STREPTOCOCCAL
RECEPTOR POLYSACCHARIDE
ACTINOMYCES

STREPTOCOCCAL
RECEPTOR POLYSACCHARIDE
ACTINOMYCES ADHESIN

Nyvad 1987

Palmer 2003
Summary

Classical bacteriology has taught us very much about the microflora of easily accessible human body sites.

Molecular taxonomy has increased that knowledge and provided a way to rapidly obtain complete community descriptions – individuals are ecosystems.

The microflora of diseased sites differs from that of healthy sites primarily in proportions of various OTUs – the community is the pathogen.

Oral biofilms are spatially differentiated multispecies communities from the earliest stages of development.

Cell-cell recognition (coaggregation) plays a role in community assembly in vivo.

It sure would be nice to analyze communities using more than 3 fluorophores..............
Acknowledgements

Paul Kolenbrander (NIDCR – retired)
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Antonie van Leeuwenhoek