El microbioma respiratori: Més enllà del cultiu

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Microbioma respiratori

- De que estem parlant?.
- El cultiu del les mostres es l’estándar, oi?.
- Quines implicacions té el considerar la microbiologia no cultivable?.

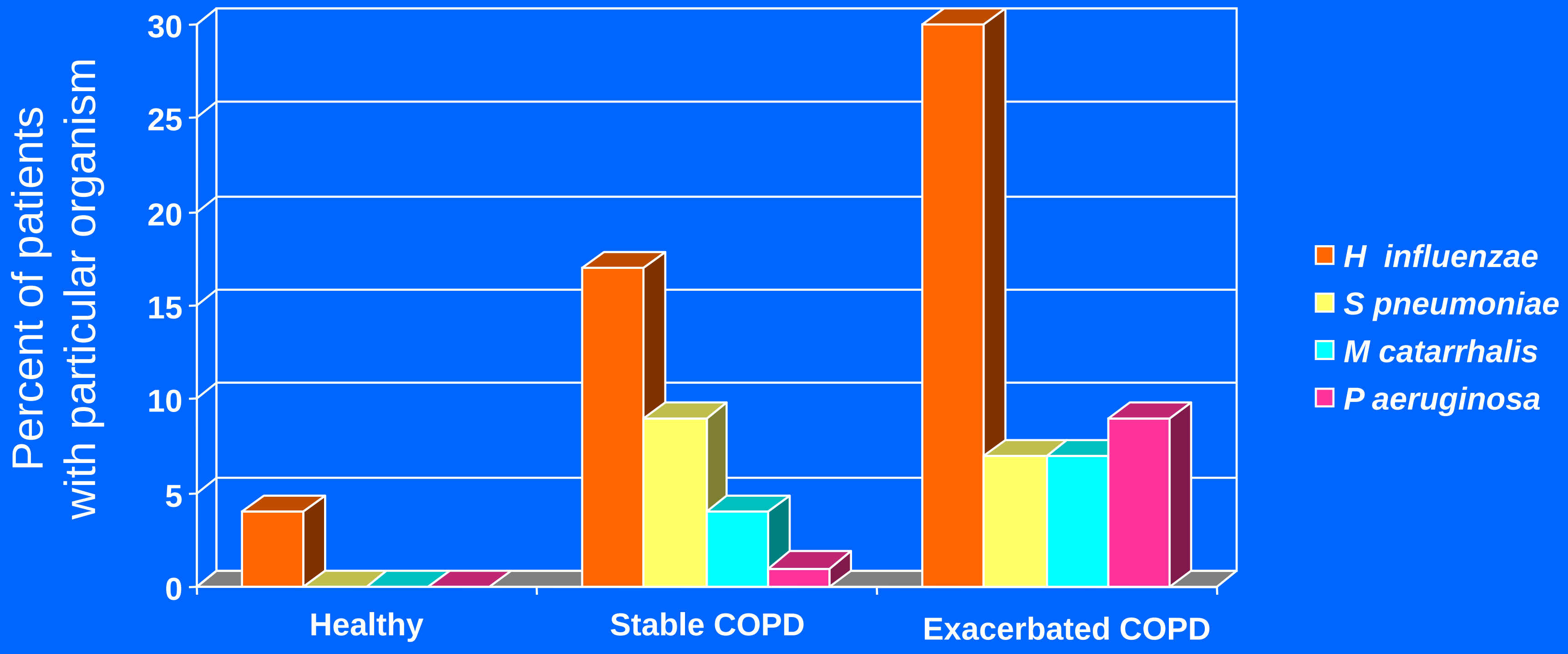
Background
COPD - Culture-based microbiology

- Sterile bronchial tree in healthy subjects.

- Colonizing PPM in COPD:
  - *H influenzae.*
  - *S pneumoniae.*
  - *M catarrhalis*.

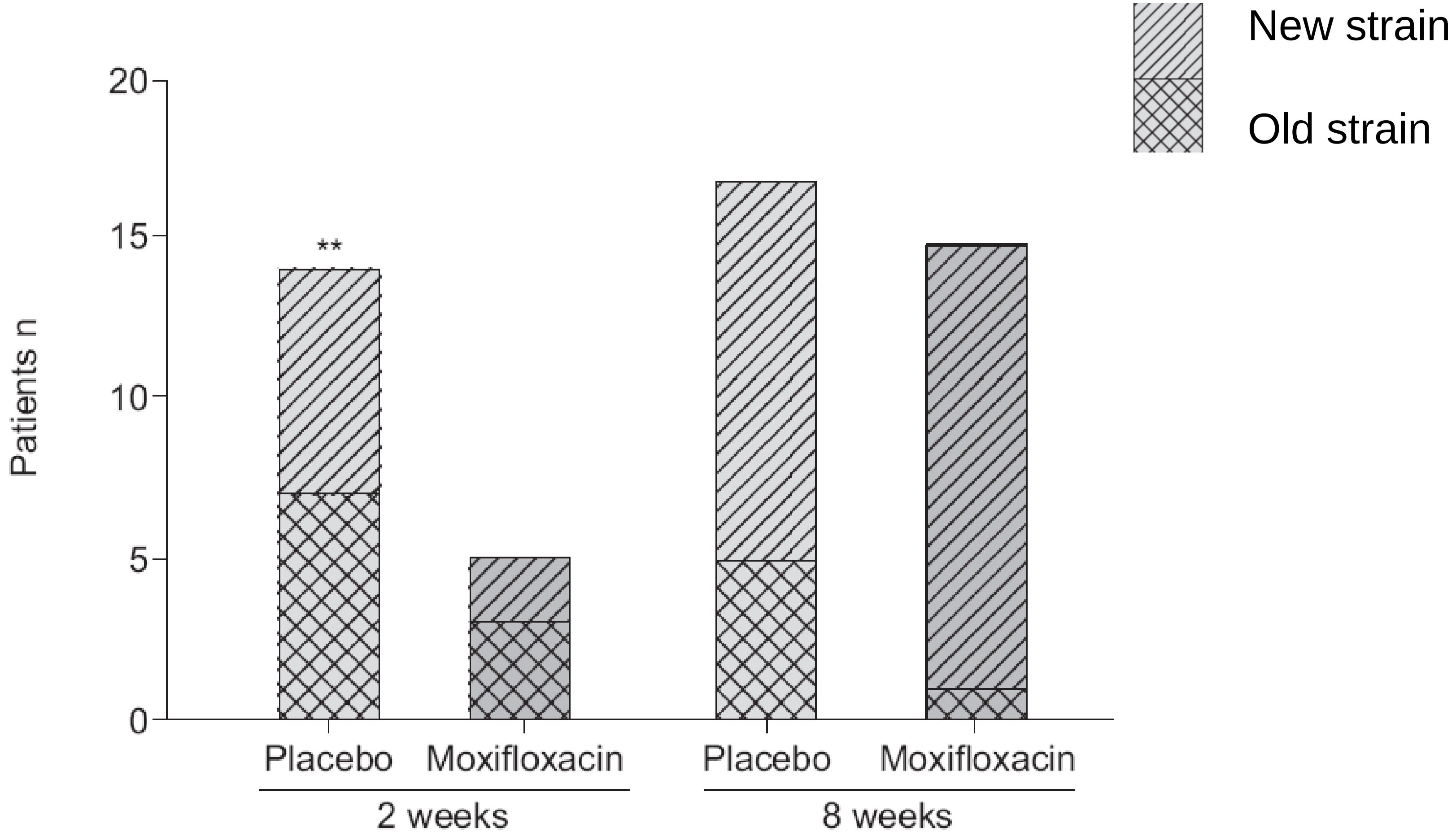
- PPM recovered stable COPD often at low load.

Bronchial colonization in COPD



Rosell. Arch Int Med 2005; 165: 891

Recurrent colonisation in COPD



Miravitlles. Eur Respir J 2009; 34:1066

New strain *H. influenzae*

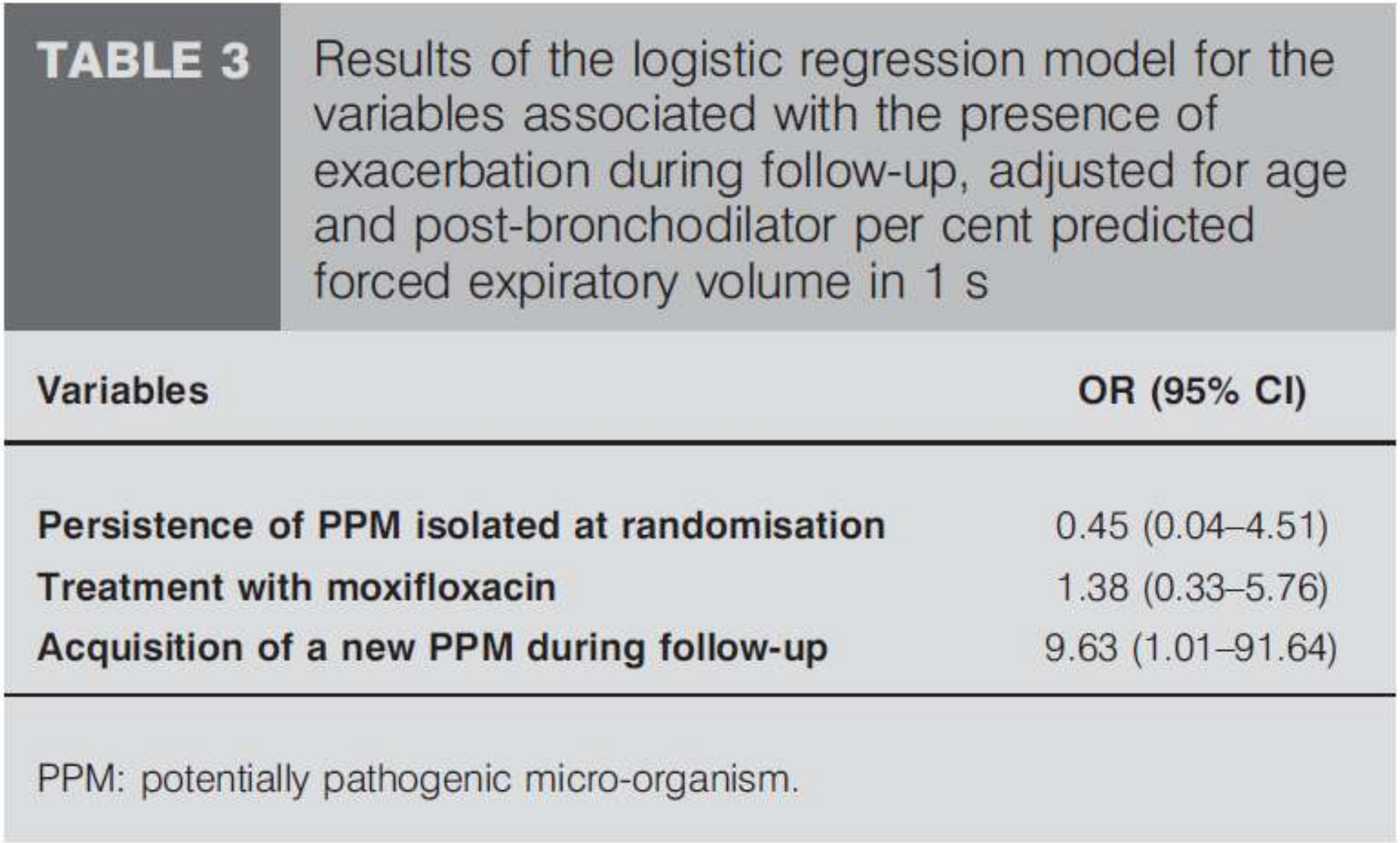


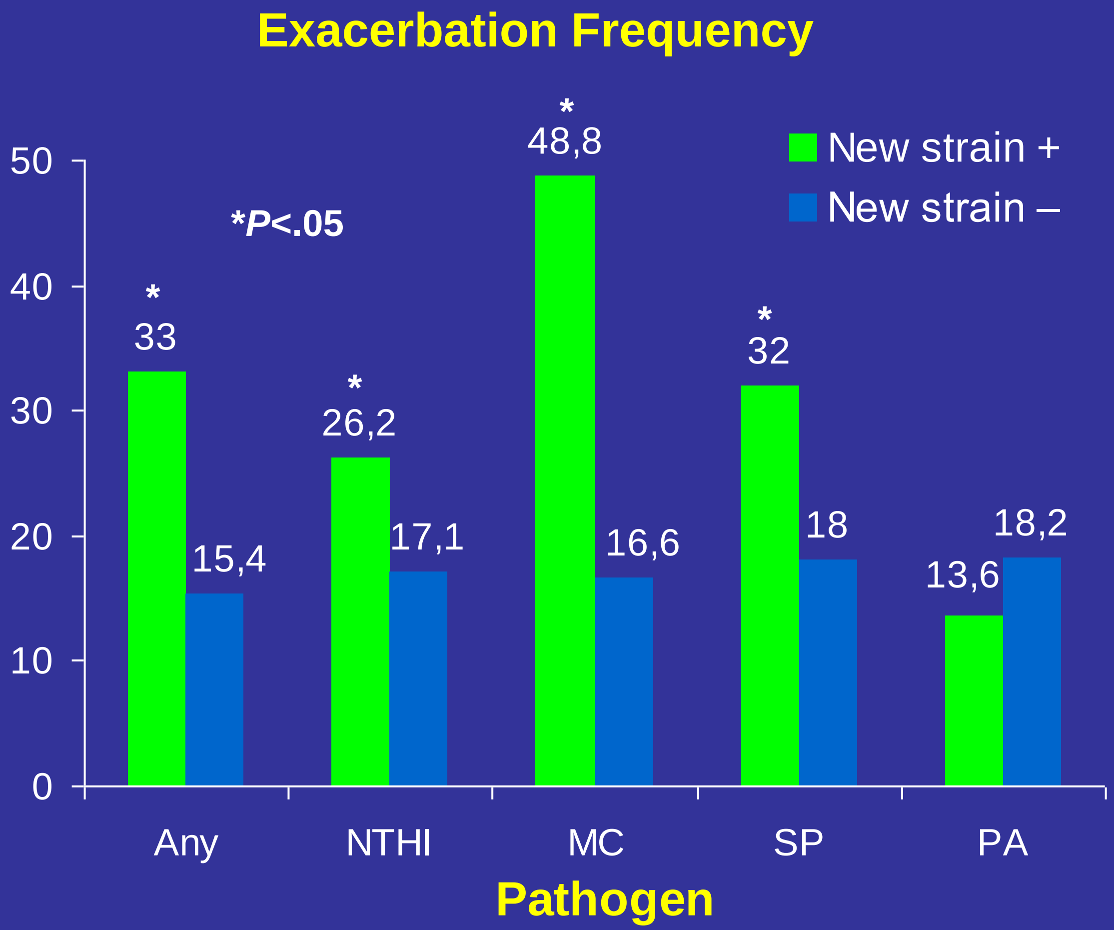
TABLE 3 Results of the logistic regression model for the variables associated with the presence of exacerbation during follow-up, adjusted for age and post-bronchodilator per cent predicted forced expiratory volume in 1 s

Variables	OR (95% CI)
Persistence of PPM isolated at randomisation	0.45 (0.04–4.51)
Treatment with moxifloxacin	1.38 (0.33–5.76)
Acquisition of a new PPM during follow-up	9.63 (1.01–91.64)

PPM: potentially pathogenic micro-organism.

Eur Respir J 2009; 34: 1066–1071

New Strain Isolation and Exacerbation

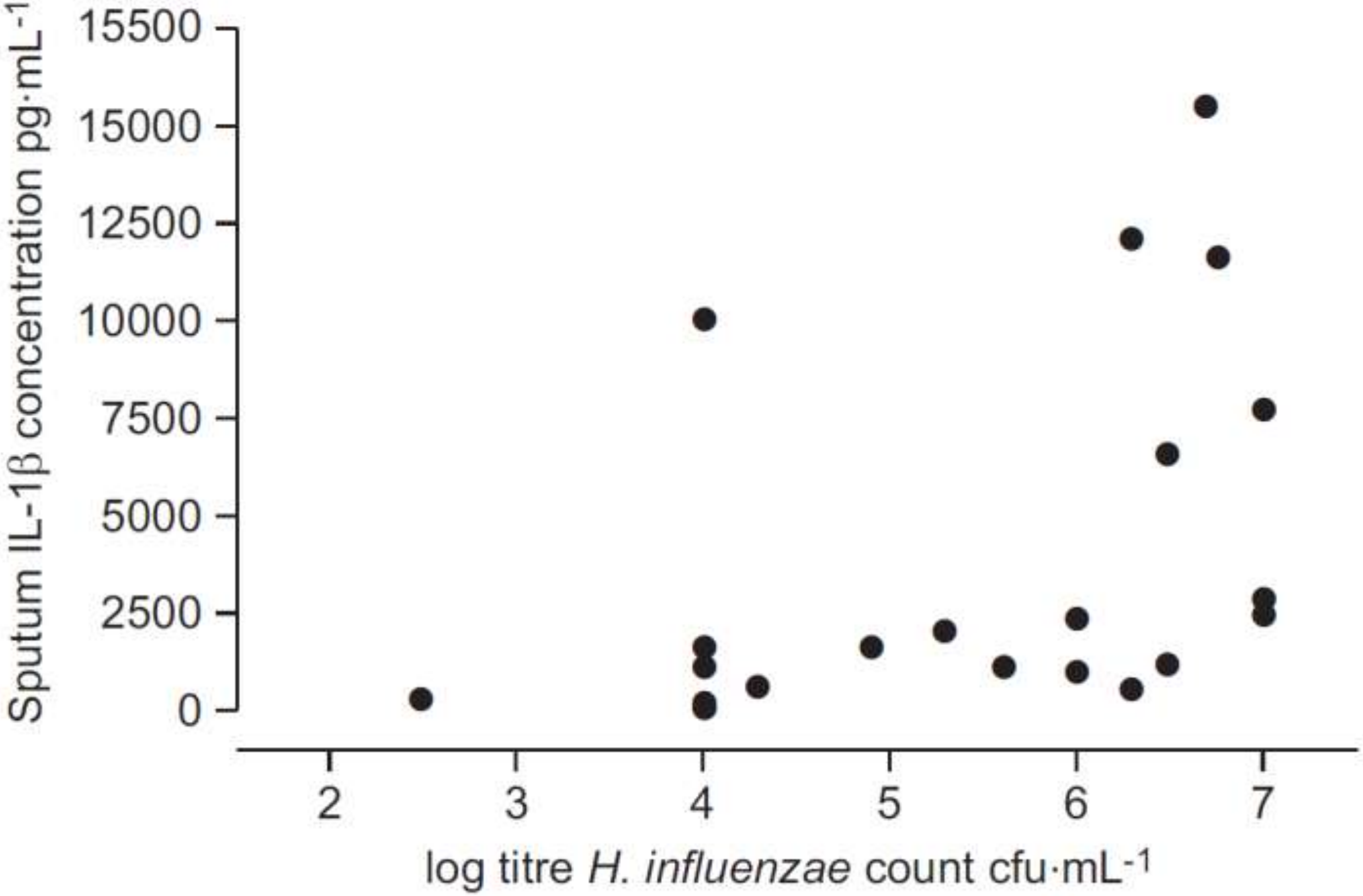


RR (95CI) exacerbation

Any	**2.15 (1.83–2.63)**
HI	**1.69 (1.37–2.09)**
MC	**2.96 (2.39–3.67)**
SP	**1.77 (1.14–2.75)**
PA	**0.61 (0.21–1.82)**

Sethi S. *N Engl J Med* 2002;347:465-471

H. influenzae and inflammation



Eur Respir J 2010; 35: 295–302

Culture-based microbiology

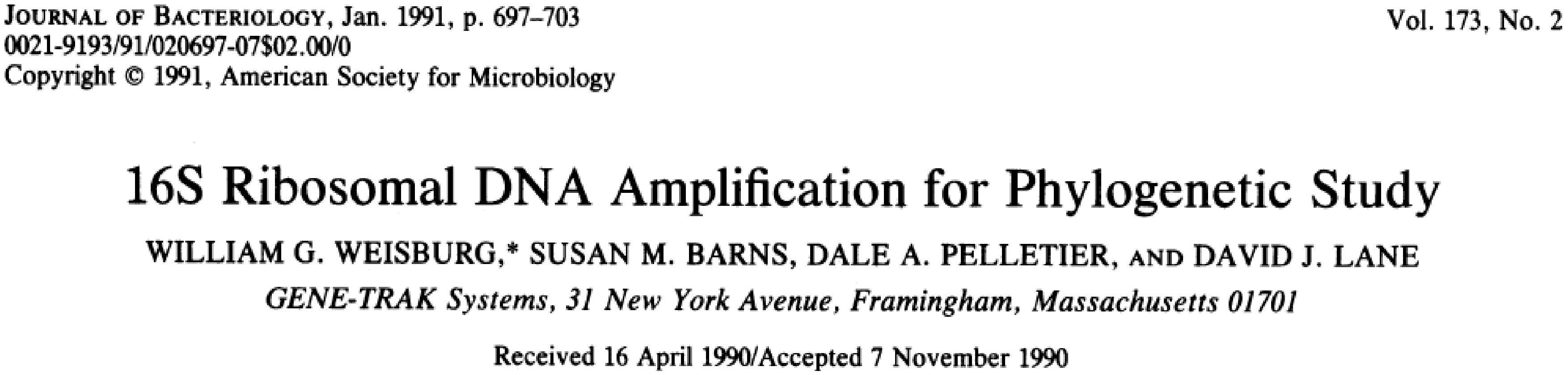
- *H. influenzae, M. catharralis, S. pneumoniae, P. aeruginosa* main pathogens causing colonization and infection.

- Bronchial colonization associated with inflammation, mainly attributable to *H. influenzae.*

~5 % of microorganisms currently culturable, depending on

Environment
Sample
Effort

Staley and Konopka *1985 Annual Review of Microbiology 39 pp 321-346*



JOURNAL OF BACTERIOLOGY, Jan. 1991, p. 697–703
0021-9193/91/020697-07$02.00/0
Vol. 173, No. 2

16S Ribosomal DNA Amplification for Phylogenetic Study

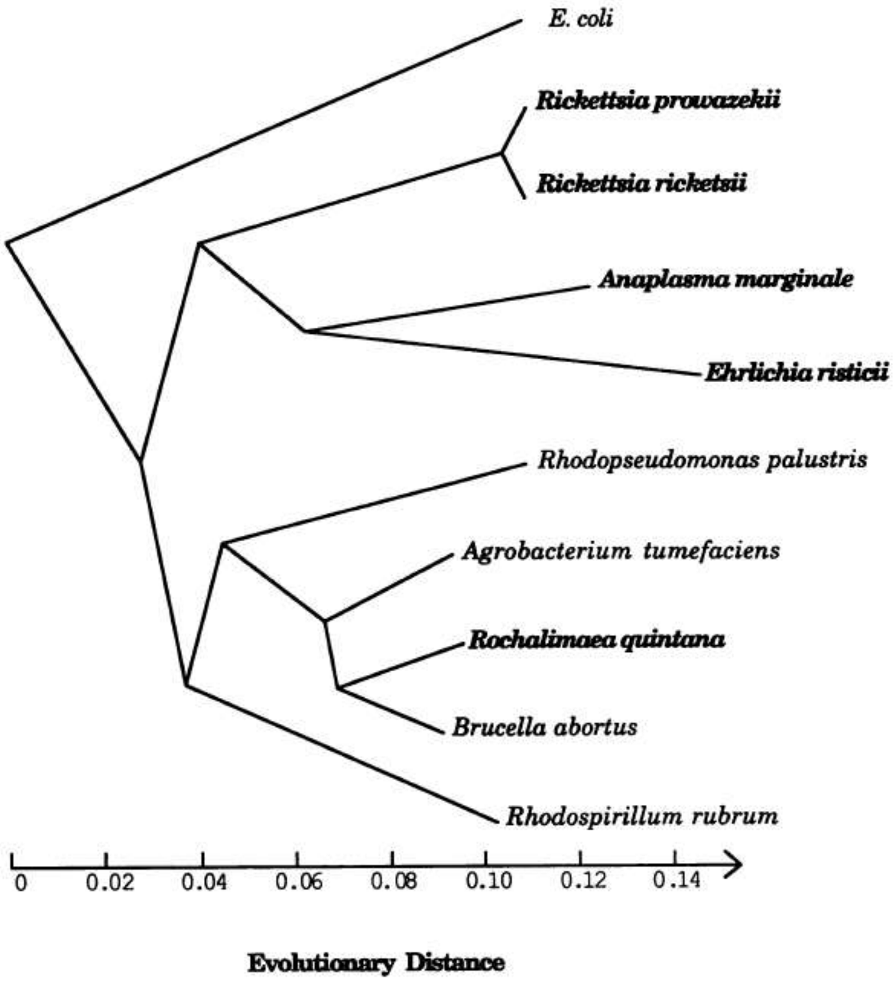
WILLIAM G. WEISBURG,* SUSAN M. BARNS, DALE A. PELLETIER, AND DAVID J. LANE
GENE-TRAK Systems, 31 New York Avenue, Framingham, Massachusetts 01701

Received 16 April 1990/Accepted 7 November 1990

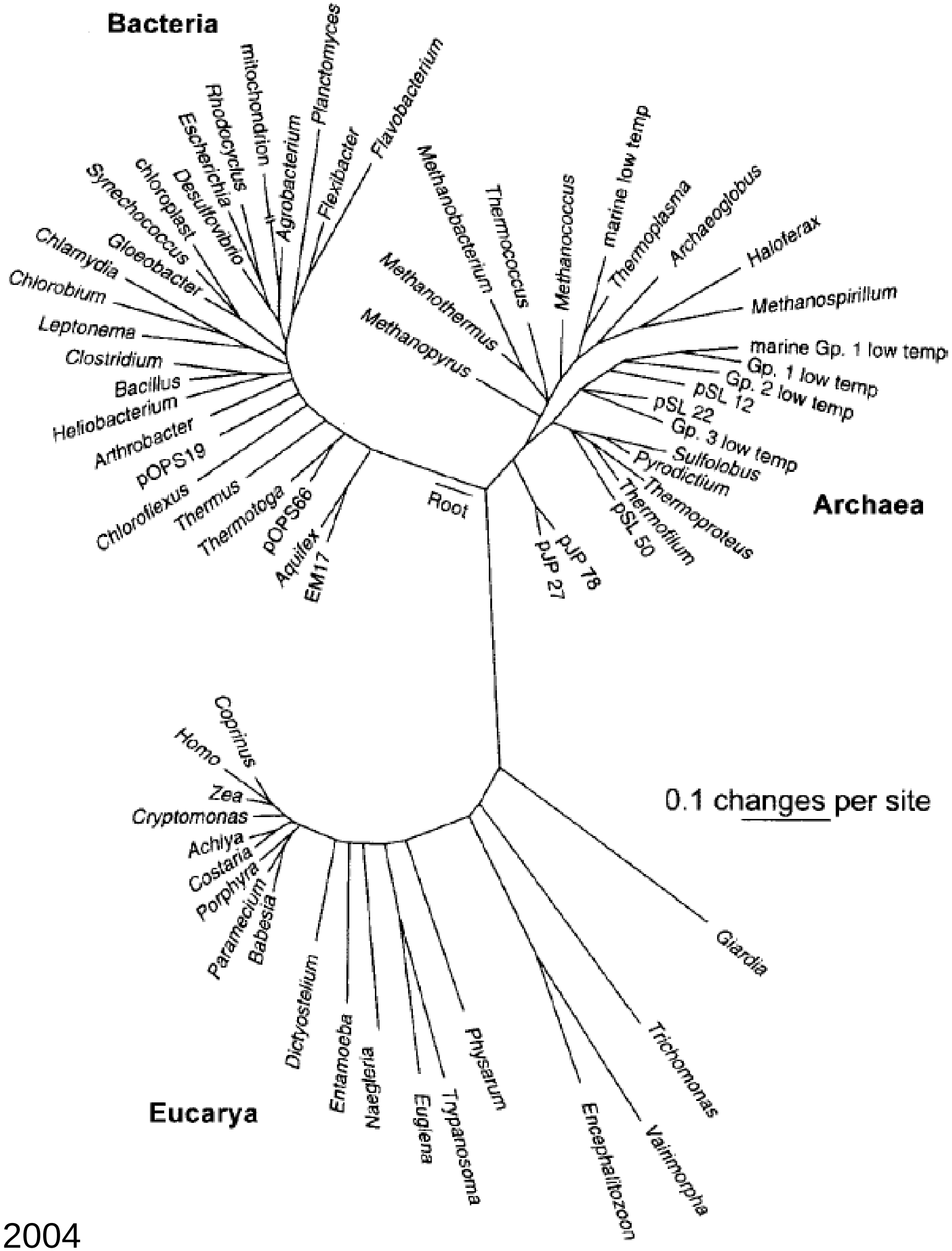
- Primers capable of amplifying nearly full-length 16S ribosomal DNA (rDNA) from many bacterial genera.

- By use of 16S rDNA the phylogenetic study of extremely fastidious or highly pathogenic bacterial species can be carried out without the need to culture.

Filogenia

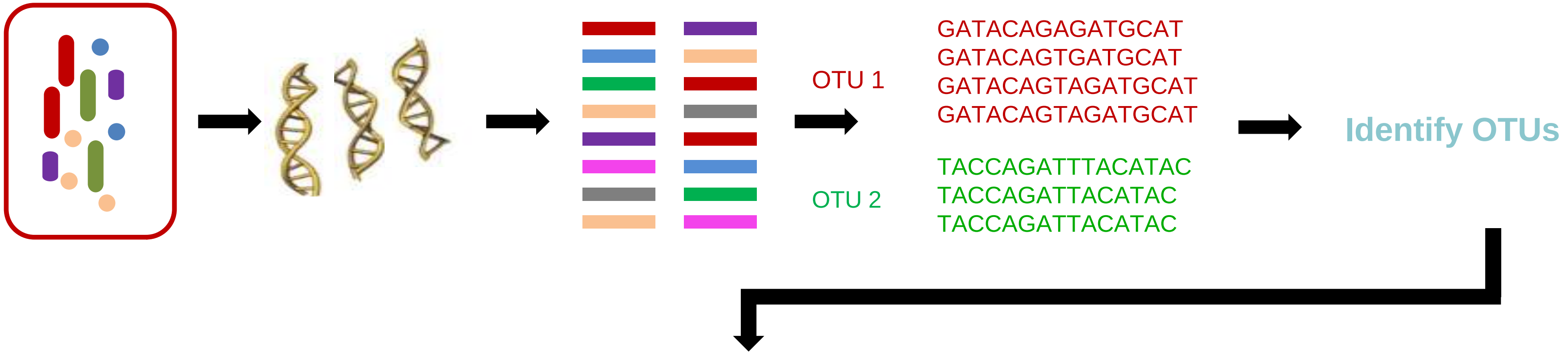

E. coli
Rickettsia prowazekii
Rickettsia ricketsii
Anaplasma marginale
Ehrlichia risticii
Rhodopseudomonas palustris
Agrobacterium tumefaciens
Rochalimaea quintana
Brucella abortus
Rhodospirillum rubrum
0
0.02
0.04
0.06
0.08
0.10
0.12
0.14
Evolutionary Distance

Universal phylogenetic tree based on 16S rRNA gene sequence comparisons



Jill E. Clarridge III
CLINICAL MICROBIOLOGY REVIEWS 2004

METHOD



Sample

Extract DNA

Amplifiy and sequence16S rRNA gene

Group sequences into Operational Taxonomic Units (OTUs)

OTU 1

GATACAGAGATGCAT
GATACAGTGATGCAT
GATACAGTAGATGCAT
GATACAGTAGATGCAT

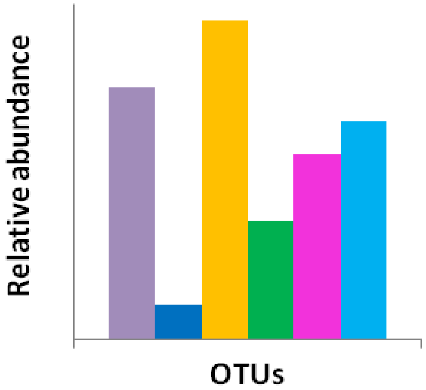
OTU 2

TACCAGATTTACATAC
TACCAGATTACATAC
TACCAGATTACATAC

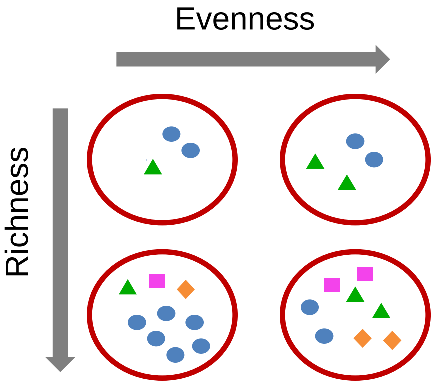
Identify OTUs

Community analysis

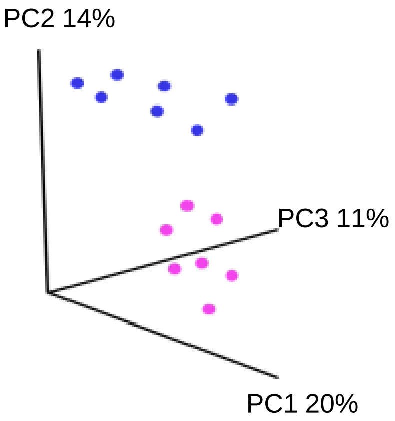
Which microorganisms are present
Relative percentage



What is the community structure



α-diversity



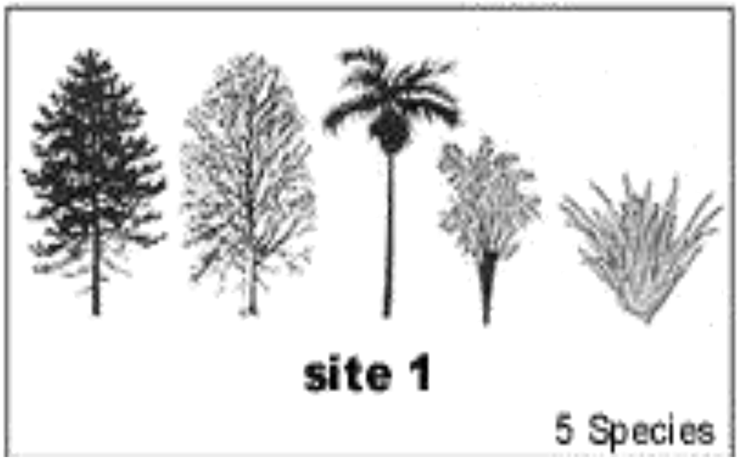
β-diversity

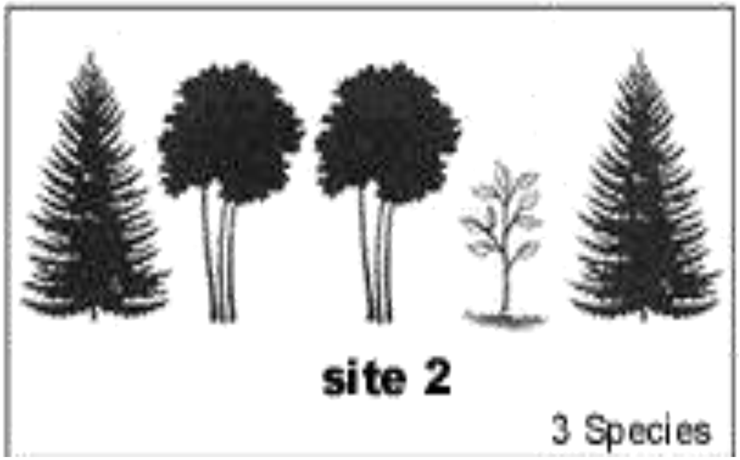
Phylogenia

Domain	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	Escherichia
Species	Escherichia coli

METHOD: alfa-diversity

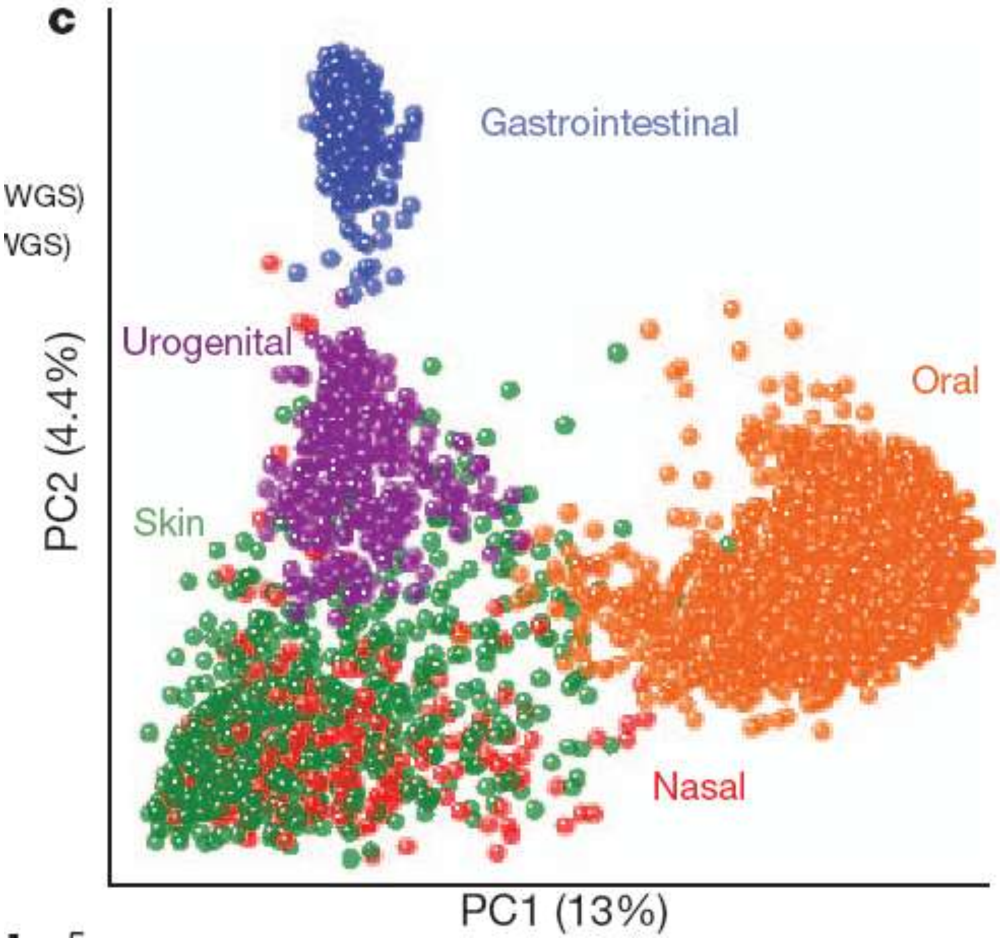
- ✓ Shannon index and Chao1 index were used as α-diversity parameters to assess both the organismal richness of a sample and the evenness of the organisms' abundance distribution in the sample
- ✓ Intra-individual measurement.





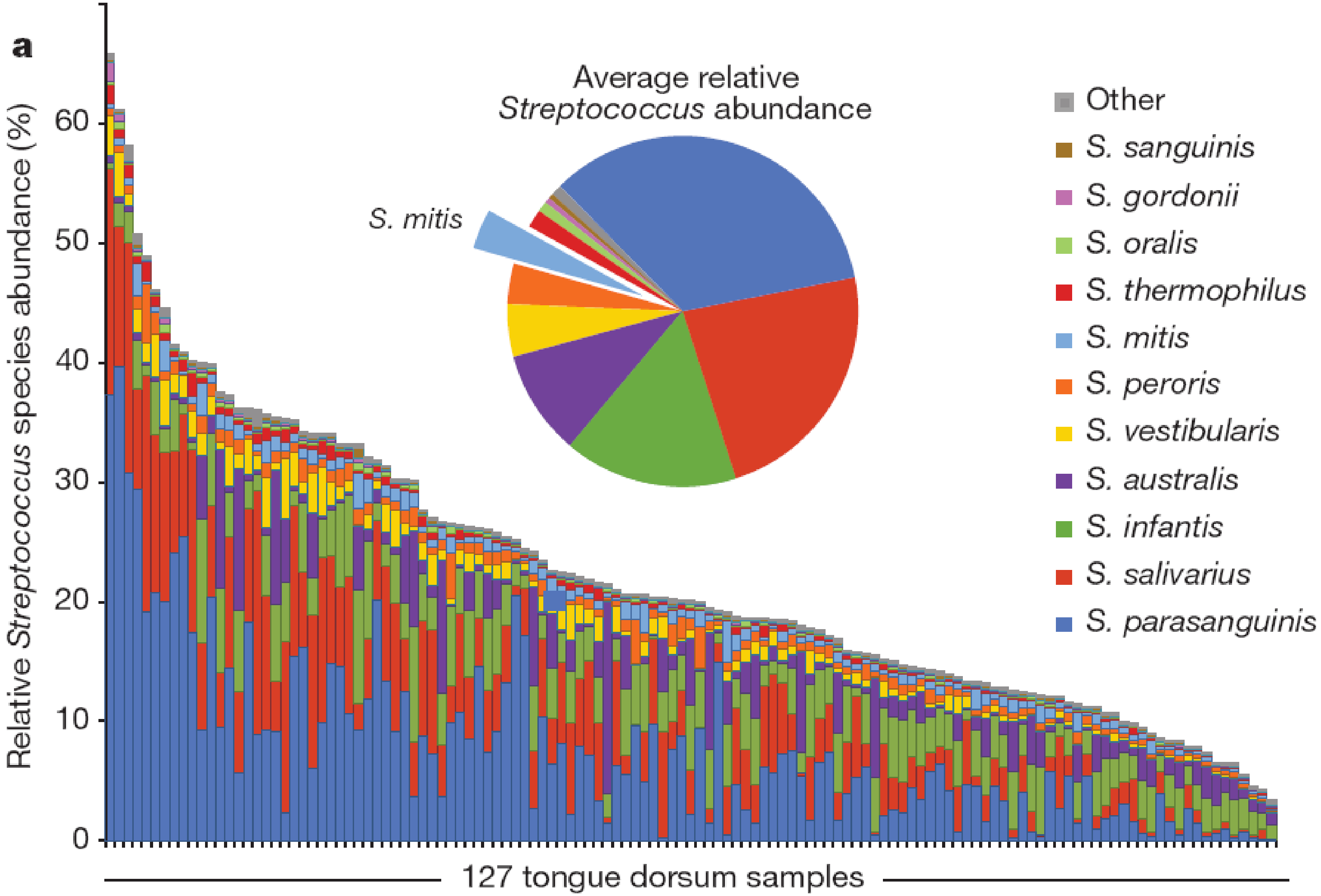
- ✓ Site 1 has higher a-diversity than site 2.

Beta diversity
Principal coordinates plot



The Human Microbiome Project Consortium. Nature 2012.

Streptococcus abundance – mouth



The Human Microbiome Project Consortium. Nature 2012.

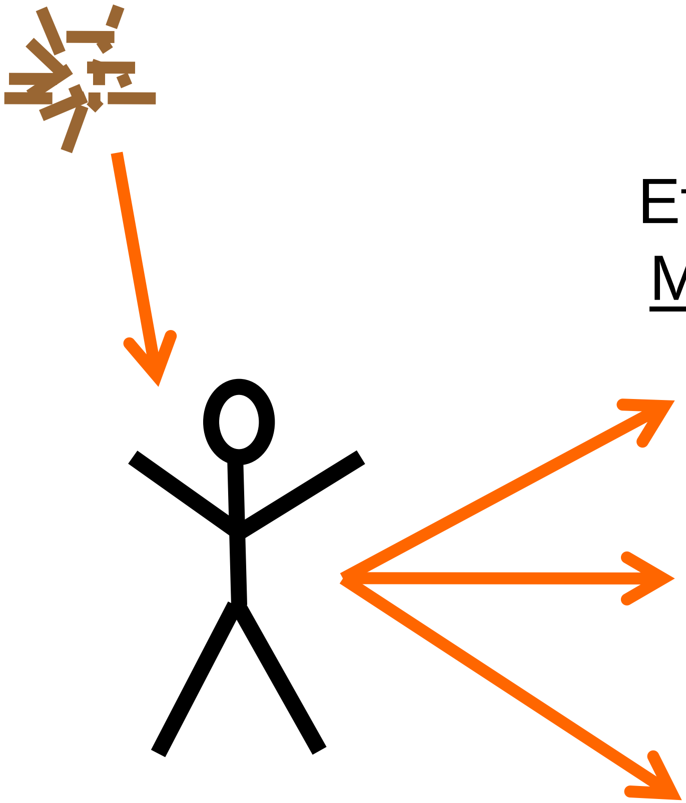
Definitions

- Microbiota/Microbiome: All the microbes that are found in a particular region or habitat.
- Taxonomy: The science of identification and clasification.
- Operational Taxonomic Unit (OTU): Specific sequences based on similarity (threshold 97%) to reference genes. Proxy for species-level divergence.
- Richness: Number different taxa in a single population.
- Alfa-Diversity: How many types of sequences in sample.
- Beta-Diversity: How many types shared among samples.

- Dysbiosis. A condition in which the normal structure of the microbiome is disturbed, through external pressures such as disease states or medication.

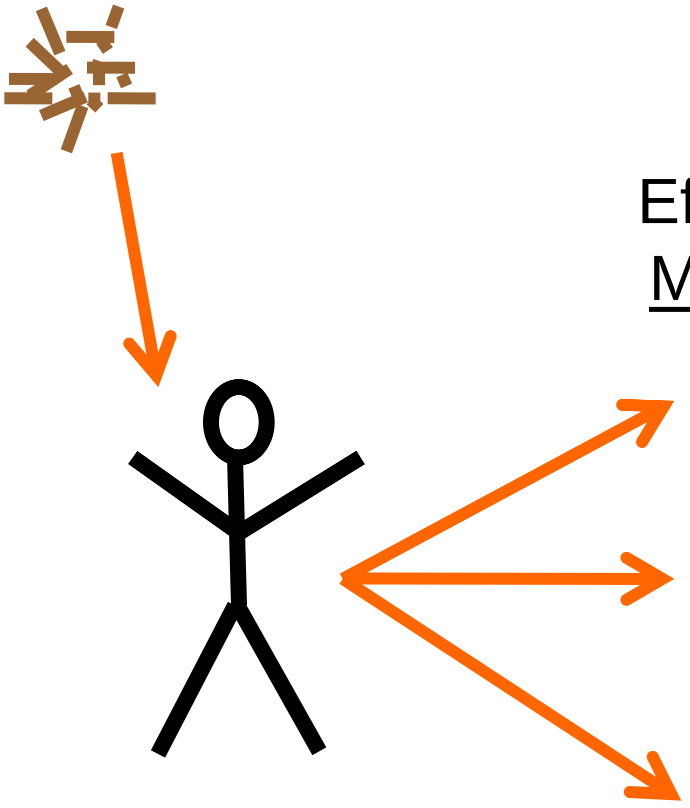
Segal. Annals ATS 2014

Colonization
Host-Microbe Symbiosis



Effect on Microbe	Effect on Host	Type of Microbial Symbiont
+	+	?
+	0	
+	–	

Symbiosis



Effect on Microbe	Effect on Host	Type of Microbial Symbiont
+	+	Mutualist
+	0	Commensal
+	–	Parasit Pathogen

Background
Culture-independent microbiology

- Molecular methods like PCR amplification of 16S rRNA gene followed by cloning and sequencing identificate many bacteria undetected by culture.

- Pyrosequencing used on PCR-amplified products from human samples add to the study of microbial diversity a high level of detail.

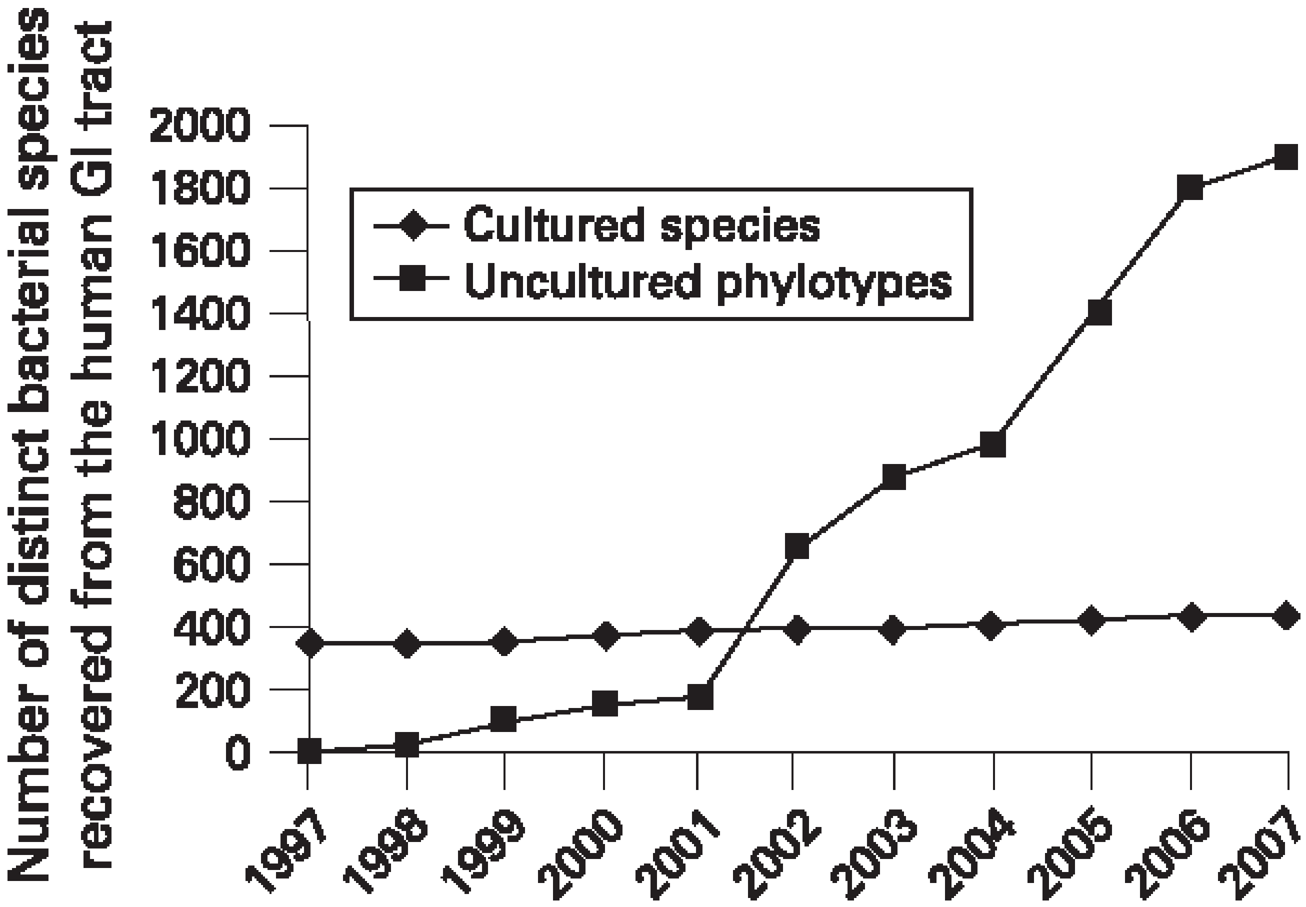
Background
Sample processing – Sequence analysis

- Ribosomal Database Project classifier:
  - Phylum, family and genus (80% confidence).
  - Taxonomically assignation >90% accuracy.

Wang Q. Appl Environ Microbiol 2007; 73:5261

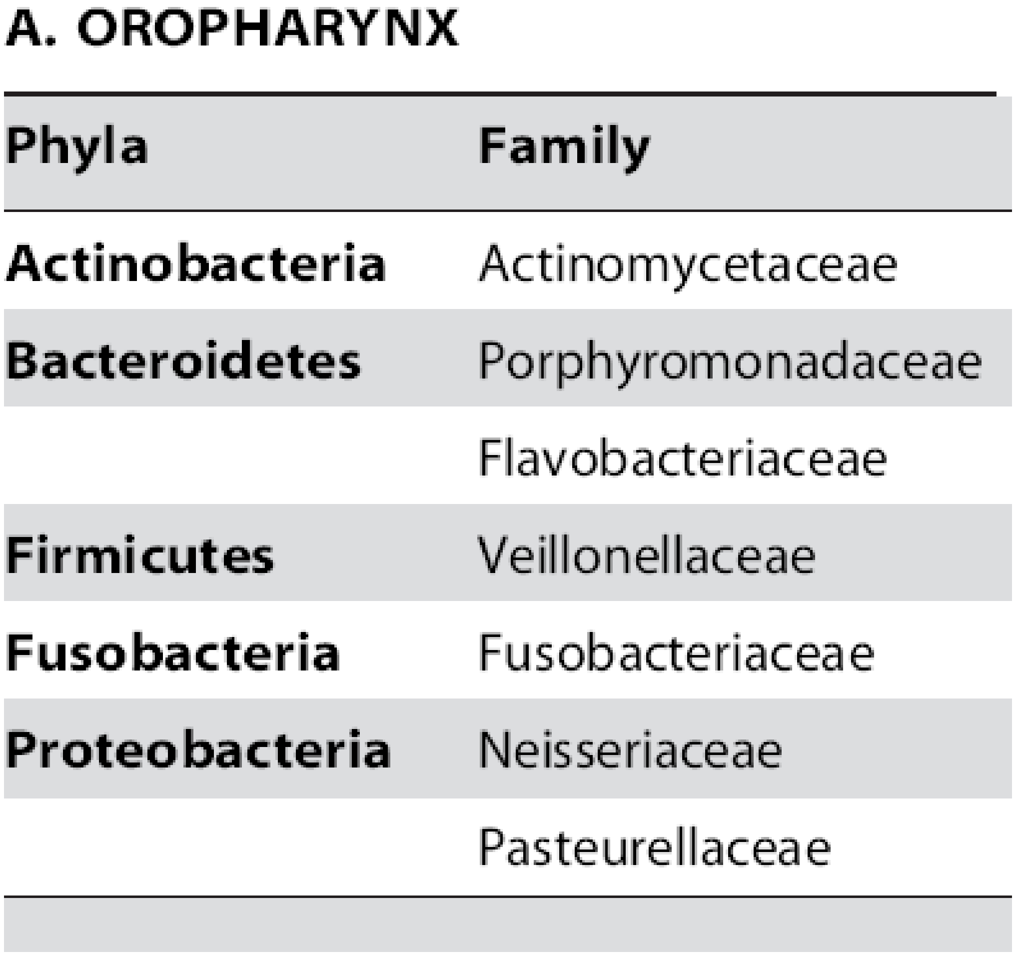
- Diversity estimation:
  - Sequences >97% identical considered same Operational Taxonomic Unit (OTUs) ~ species units.

More bacteria than imagined



Zoetendal. Gut 2008;57:1605-15

Oropharynx microbiota



A. OROPHARYNX

Phyla	Family
Actinobacteria	Actinomycetaceae
Bacteroidetes	Porphyromonadaceae
	Flavobacteriaceae
Firmicutes	Veillonellaceae
Fusobacteria	Fusobacteriaceae
Proteobacteria	Neisseriaceae
	Pasteurellaceae

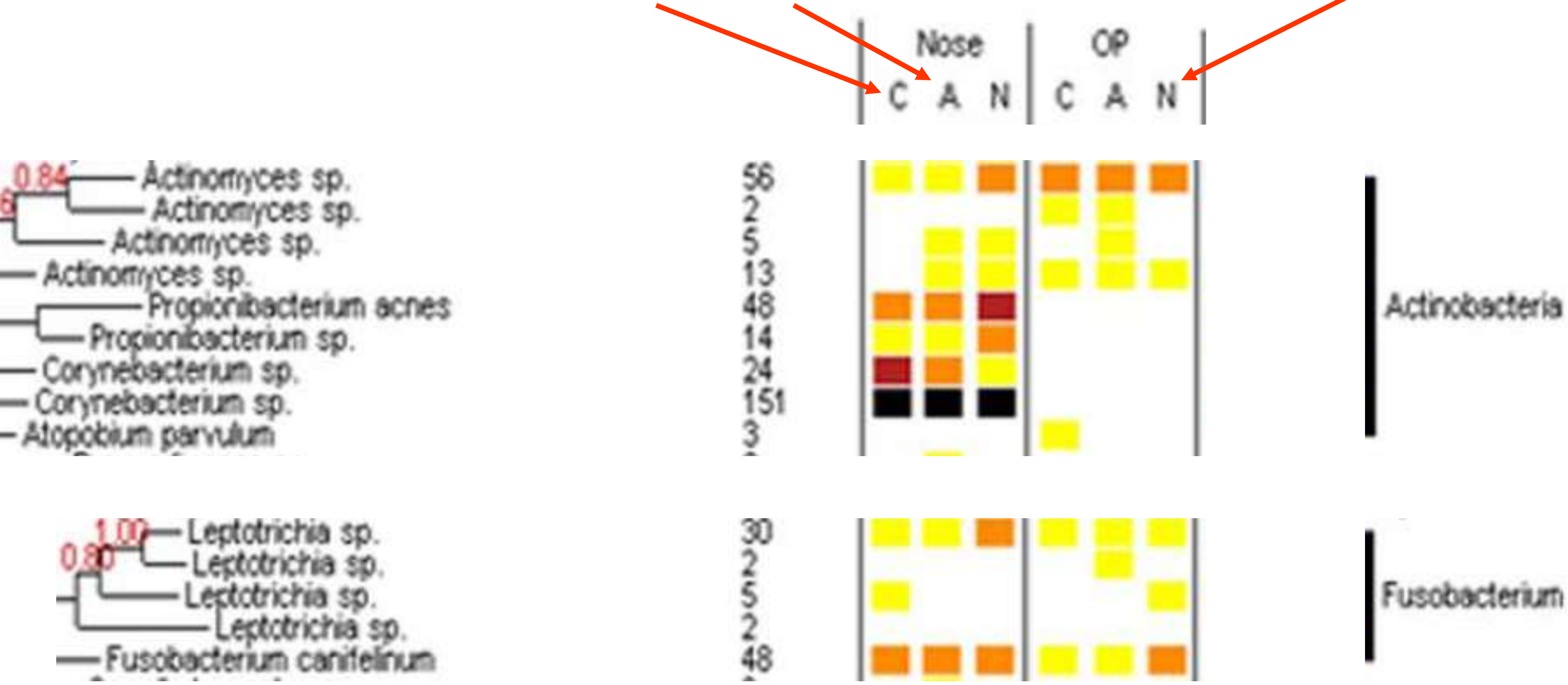
Charlson. PLoS ONE 2010 5: e15216.

Oropharyngeal flora COPD and asthma

COPD Asthma

Oropharynx

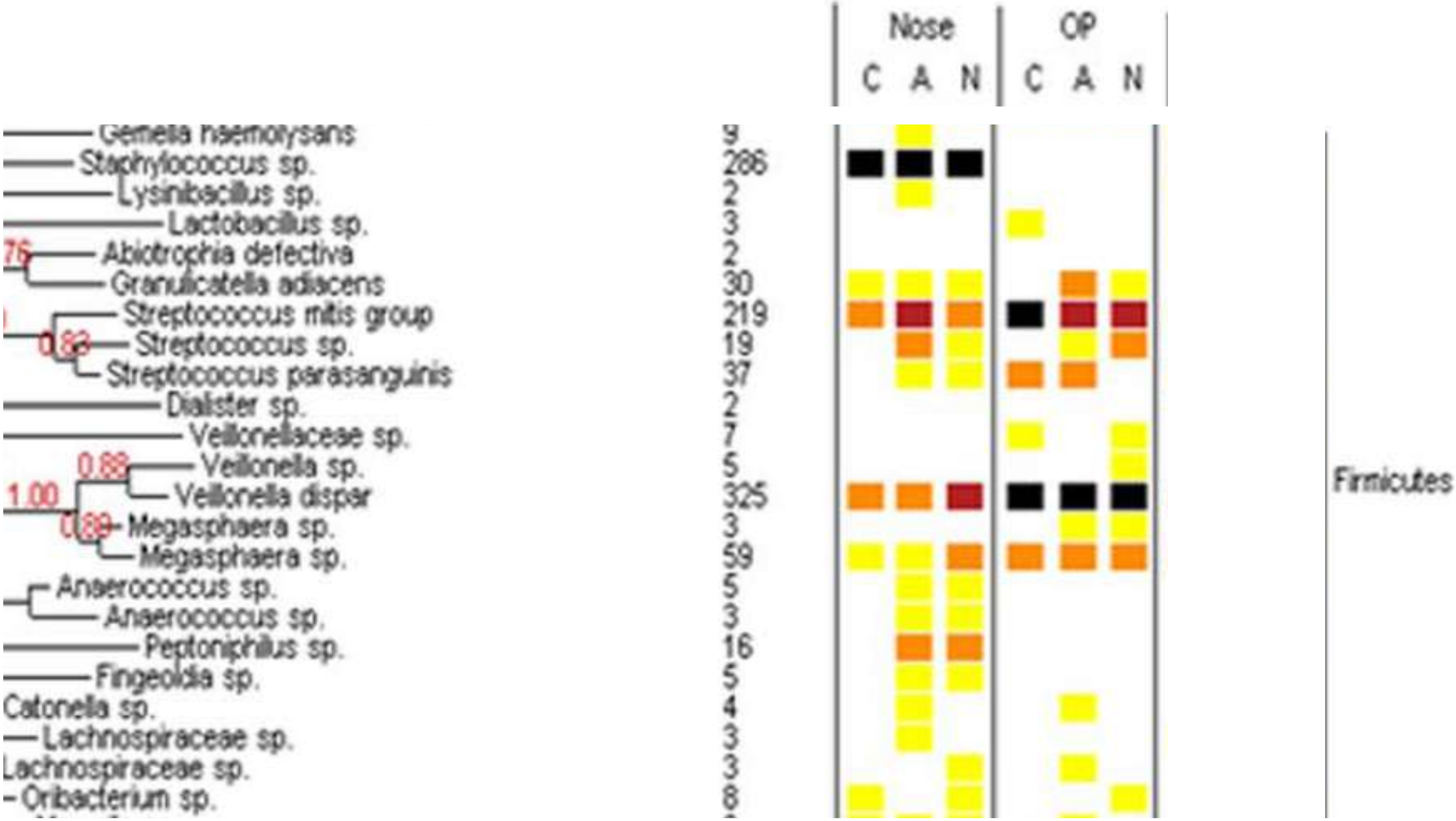
Healthy subject



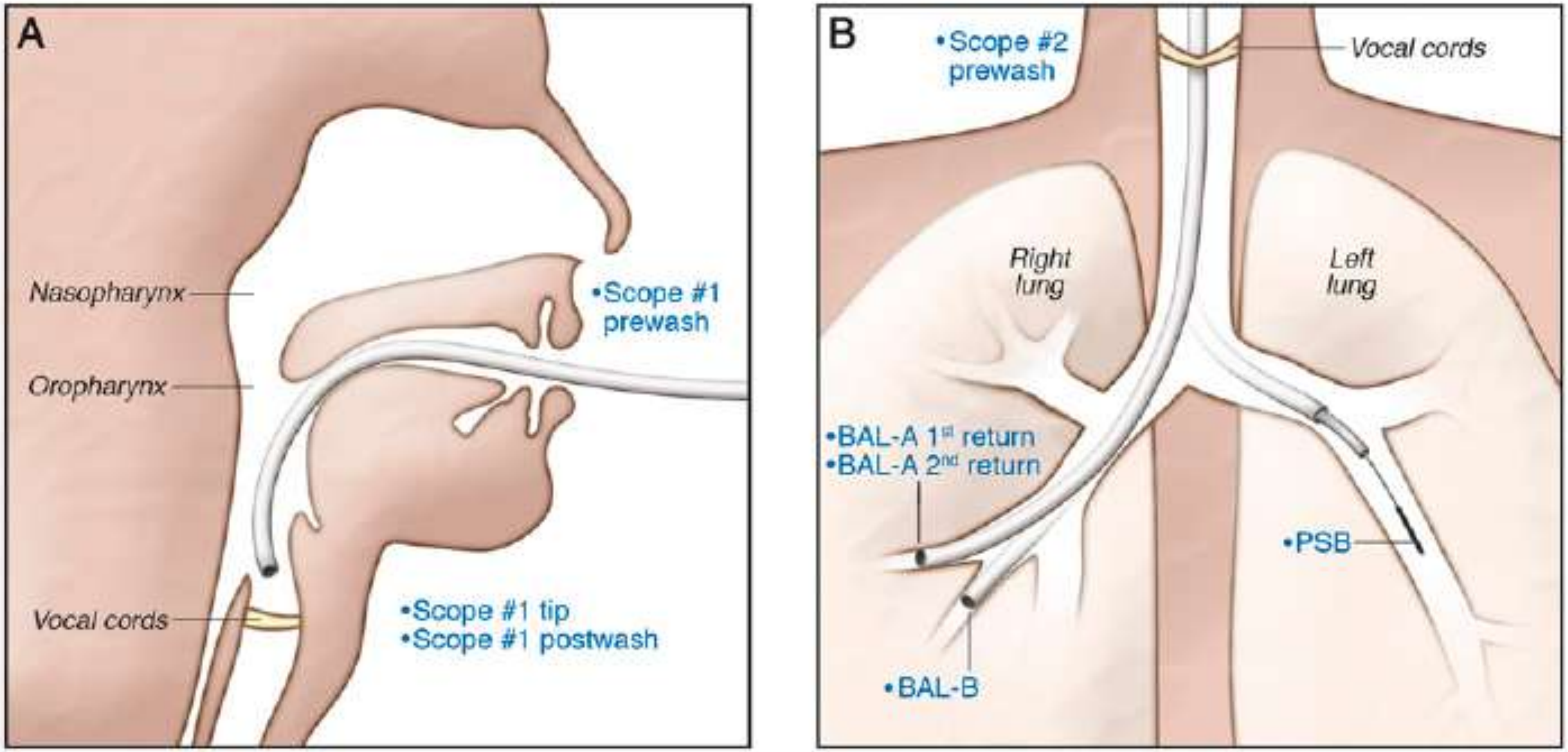
🟨 Less
⬛ More

Hilty M. PLoS ONE 2010; 5(1): e8578

Oropharyngeal flora COPD and asthma



Healthy Human Respiratory Tract



Charlson. Am J Respir Crit Care Med 20011

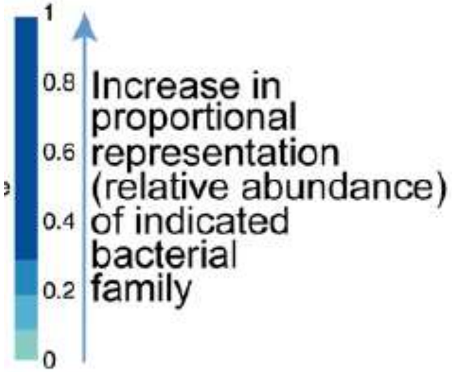
Healthy Human Respiratory Tract

Nasopharinx

Oropharinx and bronchi

Firmicutes Veillonellaceae
Bacteroidetes Prevotellaceae
Firmicutes Streptococcaceae

Similar microbioma in the oropharynx and the bronchial tree in the healthy subject

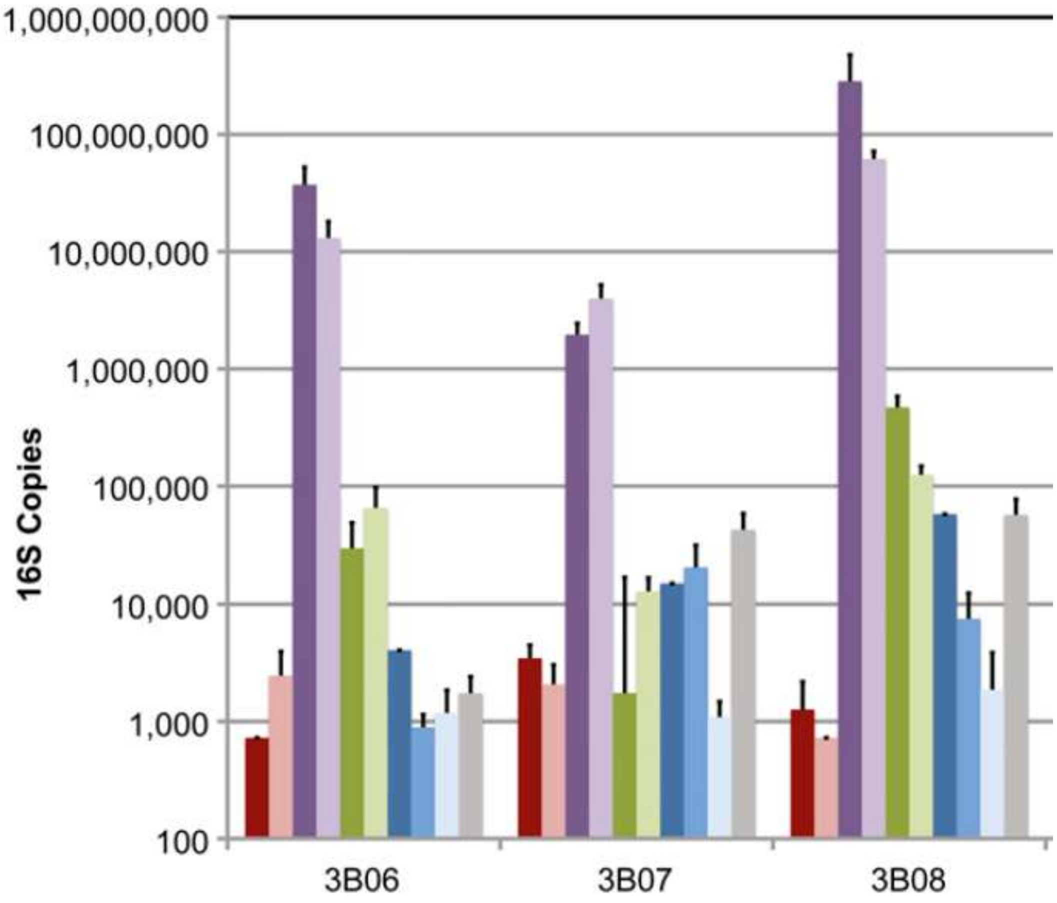


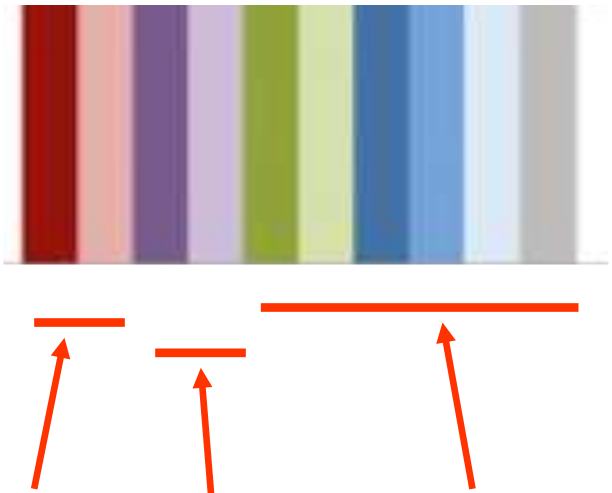
Increase in proportional representation (relative abundance) of indicated bacterial family

Firmicutes Staphylococcaceae
Actinobacteria Propionibacteriaceae

Charlson. Am J Respir Crit Care Med 20011

Healthy Human Respiratory Tract





Control

oropharynx

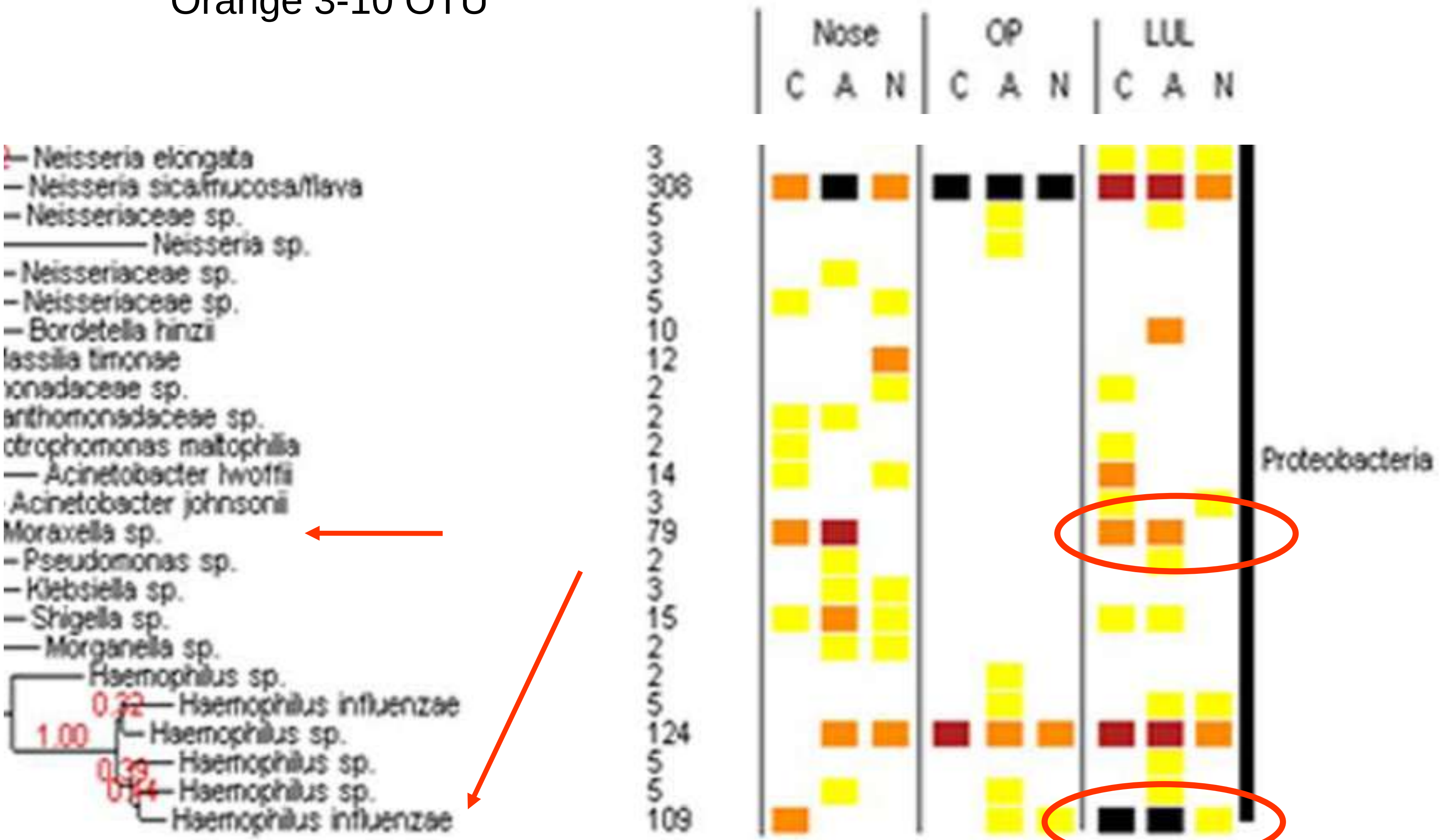
bronchial tree

Higher abundance in the oropharynx

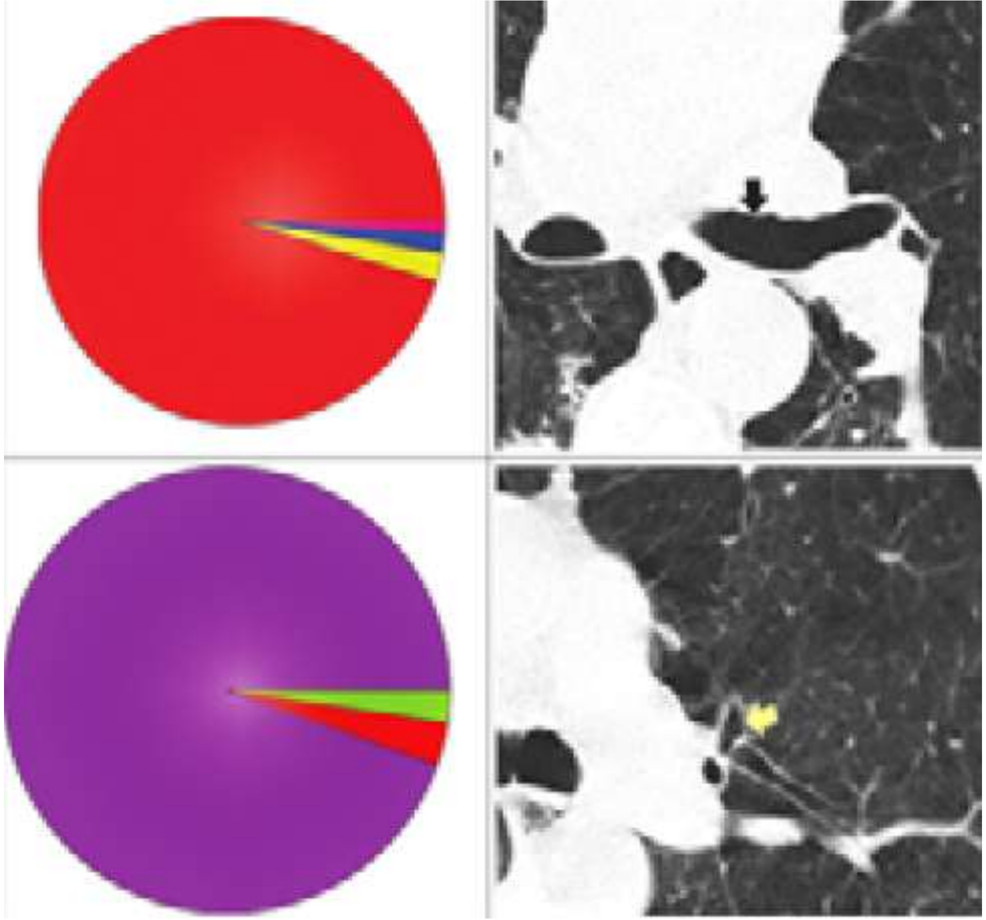
Charlson. Am J Respir Crit Care Med 20011

Proteobacteria in the bronchial tree

Red/Black >10 OTU
Orange 3-10 OTU



Severe COPD
Microbiota of the bronchi
1 cm^2 surgery

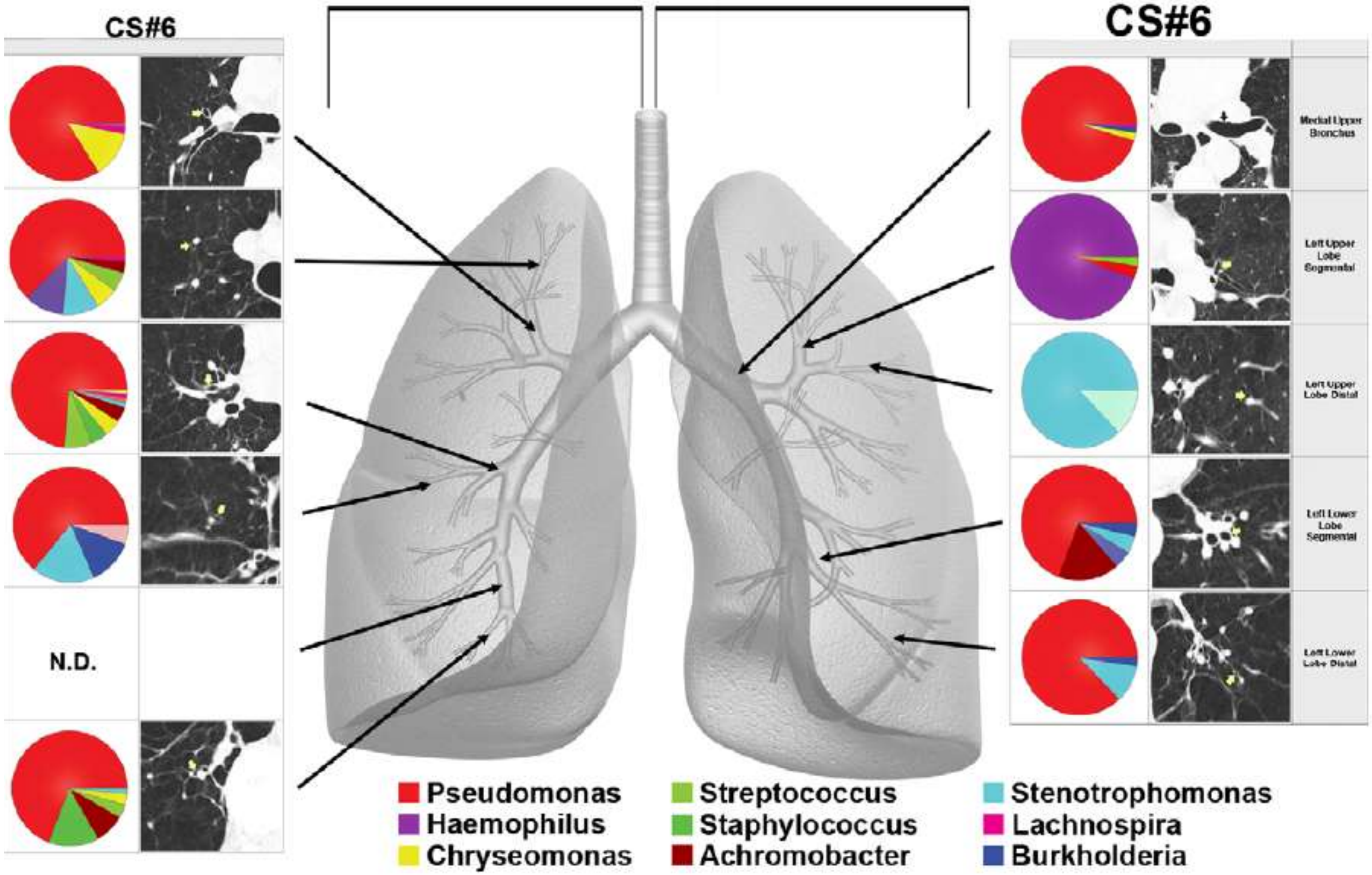


Pseudomonas

Haemophilus

Erb-Downward JR. PLoS ONE 2011; 6(2): e16384

Severe COPD
Microbiota of the bronchi



Heterogeneity of bronchial mucosa microbiota

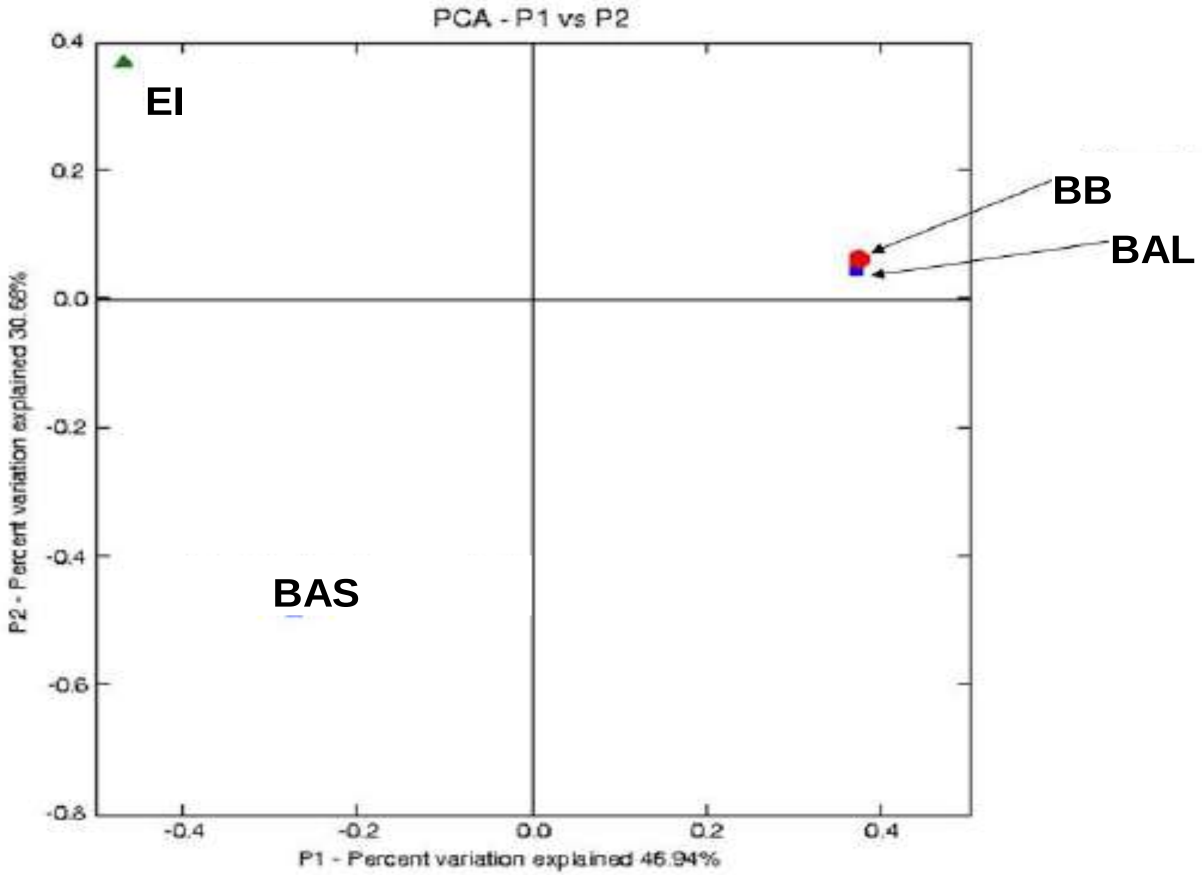
Erb-Downward JR. PLoS ONE 2011; 6(2): e16384

Background

Culture-independent microbiology

- Normal bronchial microbioma
  - Similar to oropharynx.
  - Low load.
- Chronic bronchial diseases:
  - Microbioma changes to an increase in Proteobacteria.
  - Predominance of *Haemophilus* in COPD.
  - Role of *Pseudomonas* in severe COPD.
  - Non-homogeneous in bronchi.

4 respiratory tract sample types
microbial composition - sequence of the 16S rRNA gene



Induced sputum and bronchoaspirate are different.
Bronchial biopsy and bronchoalveolar lavage cluster together.

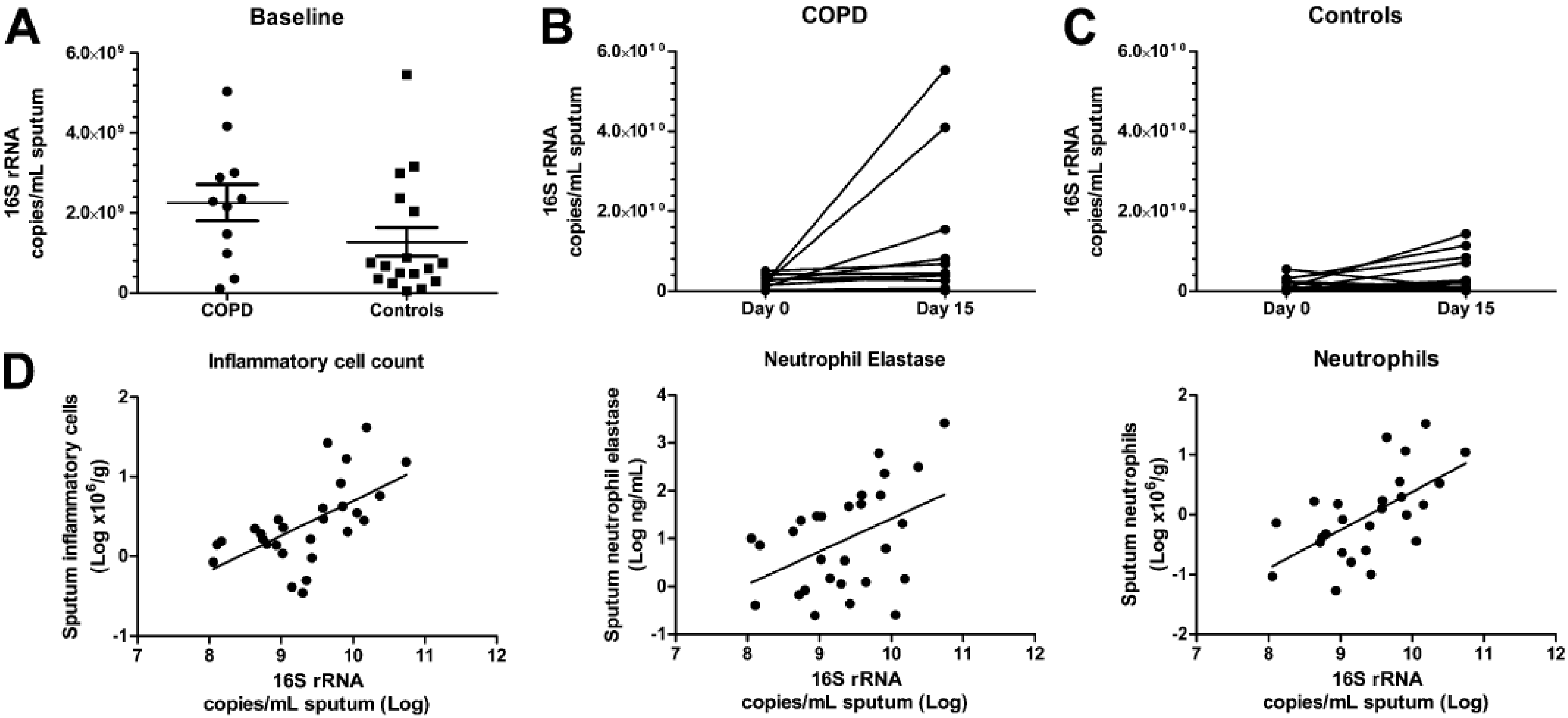
J. Clin. Microbiol. 2012, 50(11):3562

Moderate COPD
Microbiota – bronchial secretions and tissue

- Sputum and bronchial aspirate have lower diversity and a different microbiota than lower airway samples and bronchial mucosa.

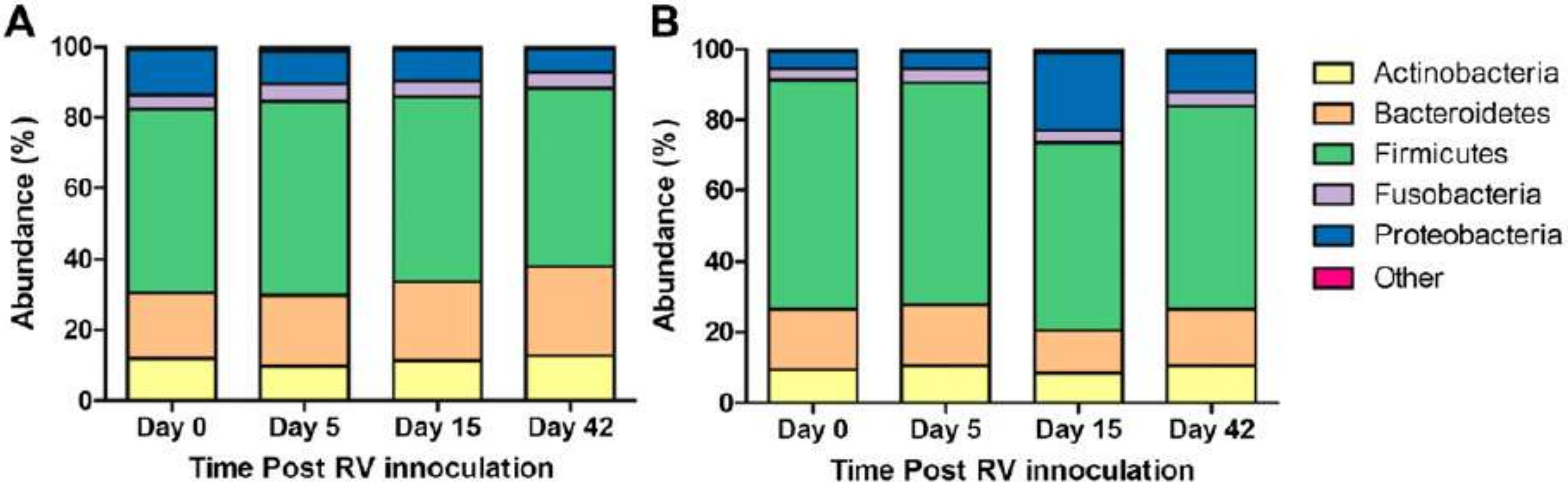
- Sputum:
  - microbial diversity >50 bacterial genera.

Effect of rhinovirus infection



Molyneaux AJRCCM 2013

Effect of rhinovirus infection



Control

COPD

Molyneaux. AJRCCM 2013

Microbioma respiratori

- De que estem parlant?.
- El cultiu del les mostres es l’estándar, oi?.
- Quines implicacions té el considerar la microbiologia no cultivable?.

Bronchial microbiome of severe COPD patients colonised by *Pseudomonas aeruginosa*

**L. Millares · R. Ferrari · M. Gallego · M. Garcia-Nuñez ·
V. Pérez-Brocal · M. Espasa · X. Pomares · C. Monton ·
A. Moya · E. Monsó**

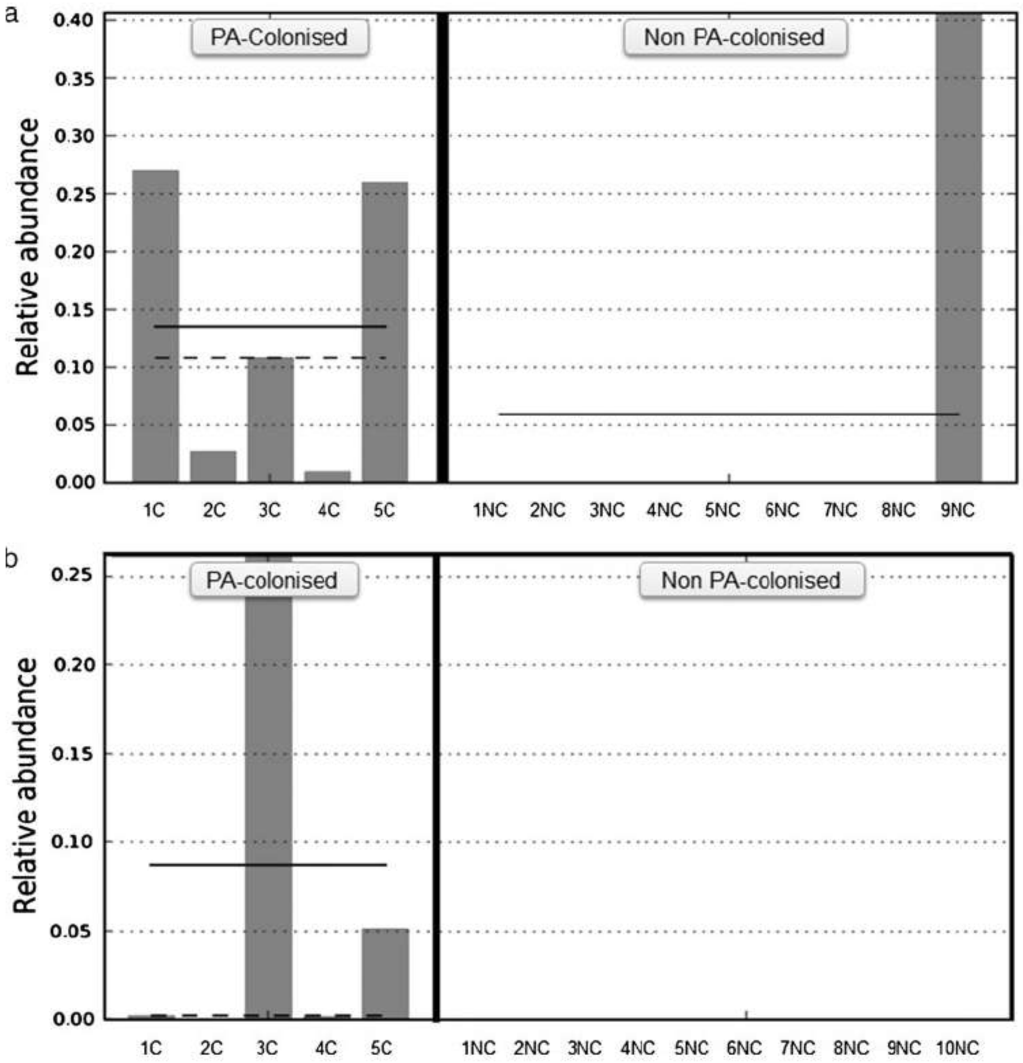
Patients	Total	PA-colonised	Non-PA-colonised
n	16	5	11
Age (years), mean (SD)	71 (6)	72 (7)	70.5 (6)
Male, *n* (%)	16 (100)	5 (100)	11 (100)
Smoking (pack-years), median (IQR)	57 (57–110)	100 (50–110)	50 (40–80)
FEV_1 post-BD (% predicted), mean (SD)	36 (30–40)	41 (30–48)	34 (30–37)
Dyspnoea scale, mean (SD)	2.25 (0.6)	2.2 (0.4)	2.3 (0.6)
BMI (kg/m^2), mean (SD)	28 (4)	28 (4)	28 (4)
6MWD (m), median (IQR)	240 (140–305)	300 (220–310)	230 (115–292)
BODE index, median (IQR)	5 (4.5–7.5)	5 (4–5)	6 (5–8)
Inhaled β2-adrenergics, *n* (%)	16 (100)	5 (100)	11 (100)
Inhaled anticholinergics, *n* (%)	16 (100)	5 (100)	11 (100)
Inhaled corticosteroids, *n* (%)	16 (100)	5 (100)	11 (100)
Positive PPM culture in stability, *n* (%)	12 (86)	5 (100)	7 (64)
Chronic colonisation by *P. aeruginosa*, *n* (%)	5 (31)	5 (100)	0

Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-013-2044-0

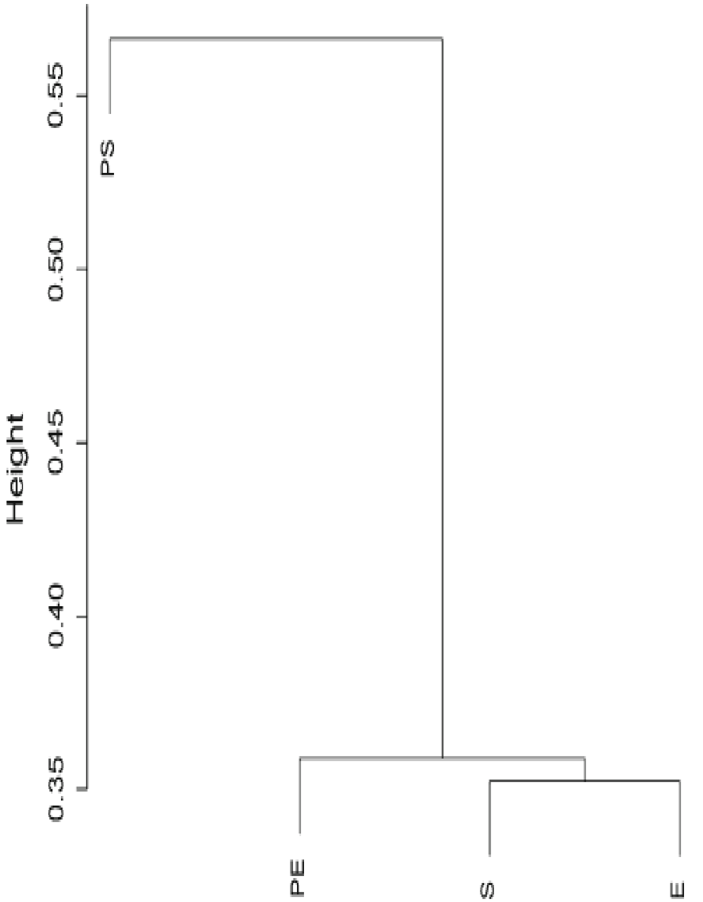
Table 2 Sputum cultures under stability and exacerbation

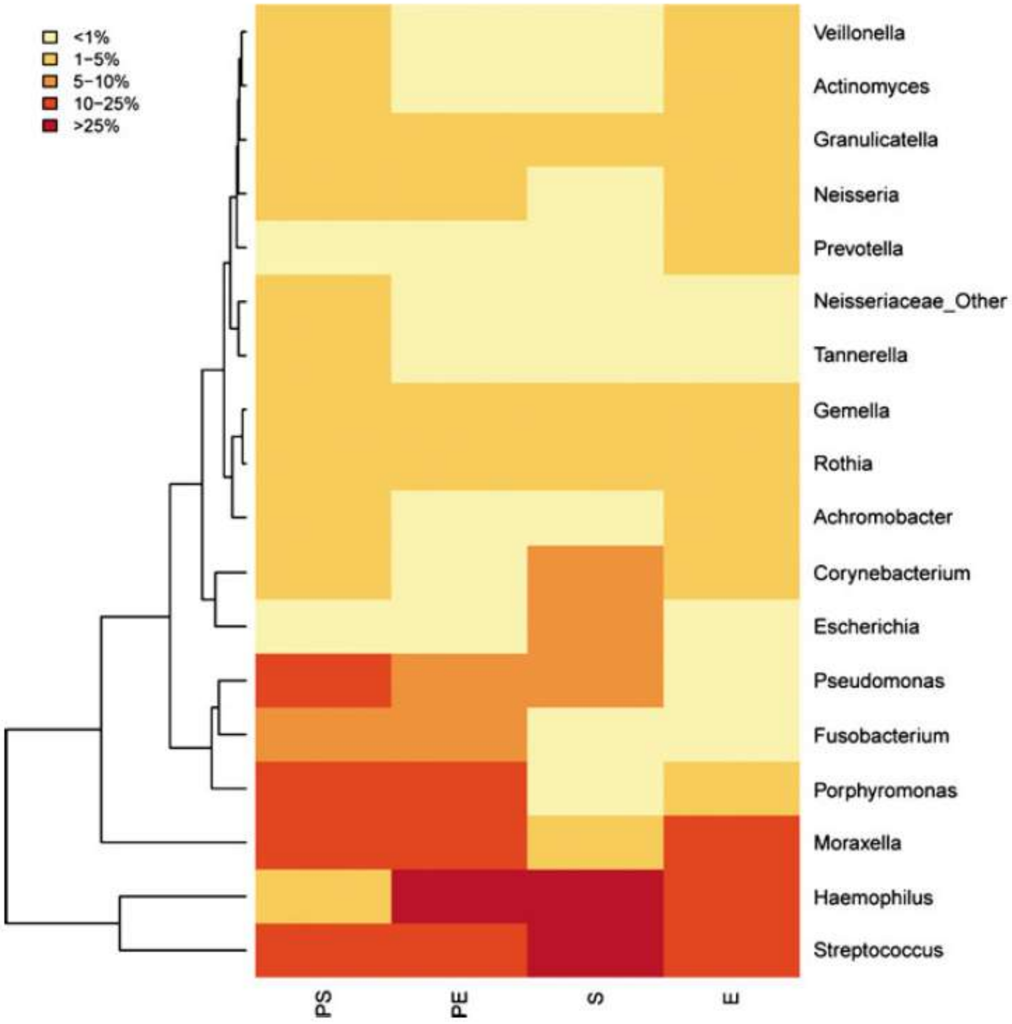
	Stable	Exacerbated
n	14	15
Positive culture for PPM, *n* (%)	12 (86)	10 (67)
Polymicrobial cultures, *n* (%)	4 (29)	3 (20)
Microorganisms		
Pseudomonas aeruginosa	5 (42)	3 (30)
Haemophilus influenza	4 (33)	4 (40)
Moraxella catarrhalis	2 (17)	1 (10)
Streptococcus pneumonia	2 (17)	1 (10)
Escherichia coli	1 (8)	–
Alcaligenes spp.	1 (8)	1 (10)
Staphylococcus aureus	–	1 (10)
Haemophilus parainfluenzae	–	1 (10)
Stenotrophomonas maltophilia	–	1 (10)

Pseudomonas relative abundance Stability and exacerbation

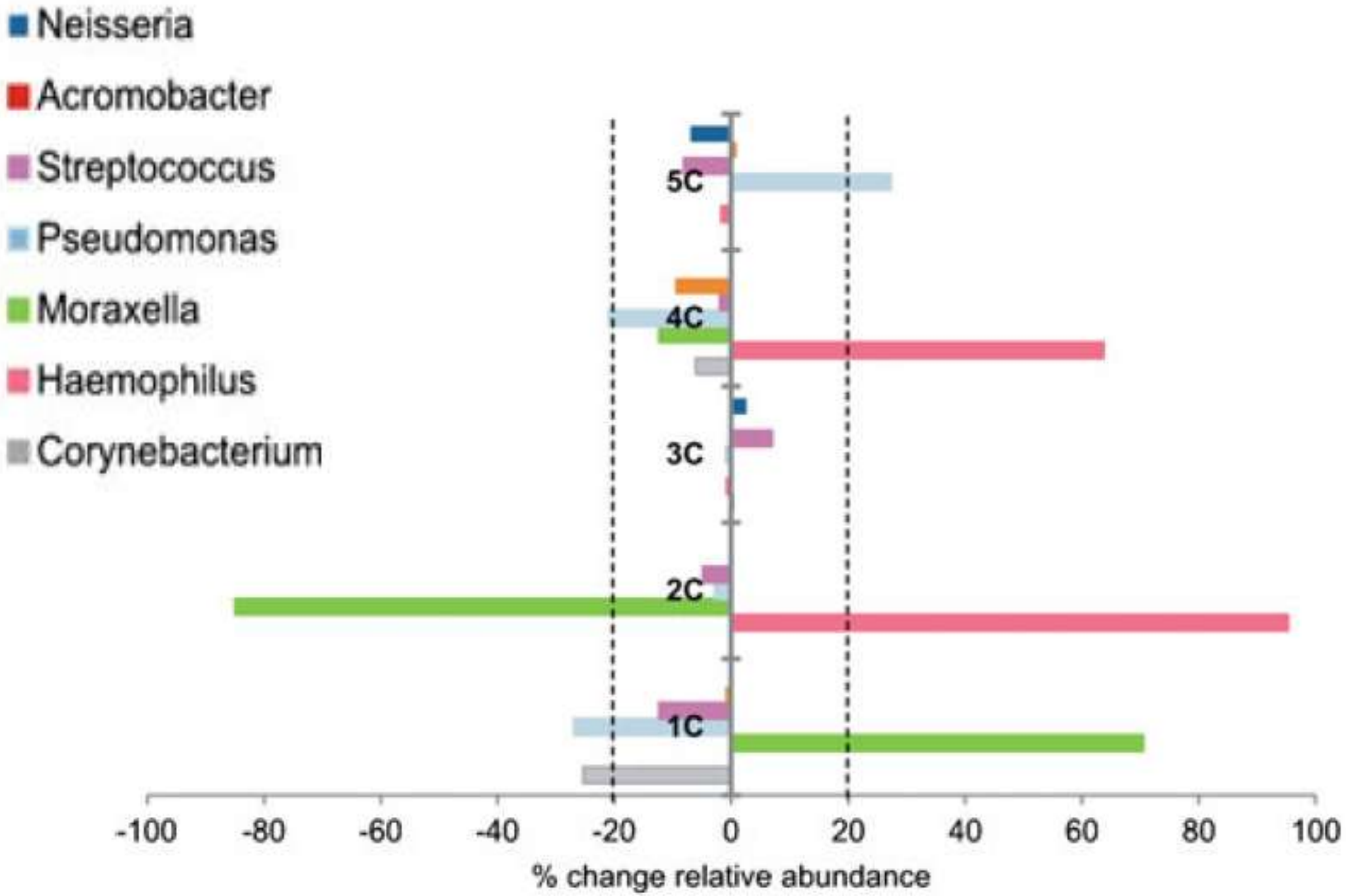


Severe COPD
stability and exacerbation

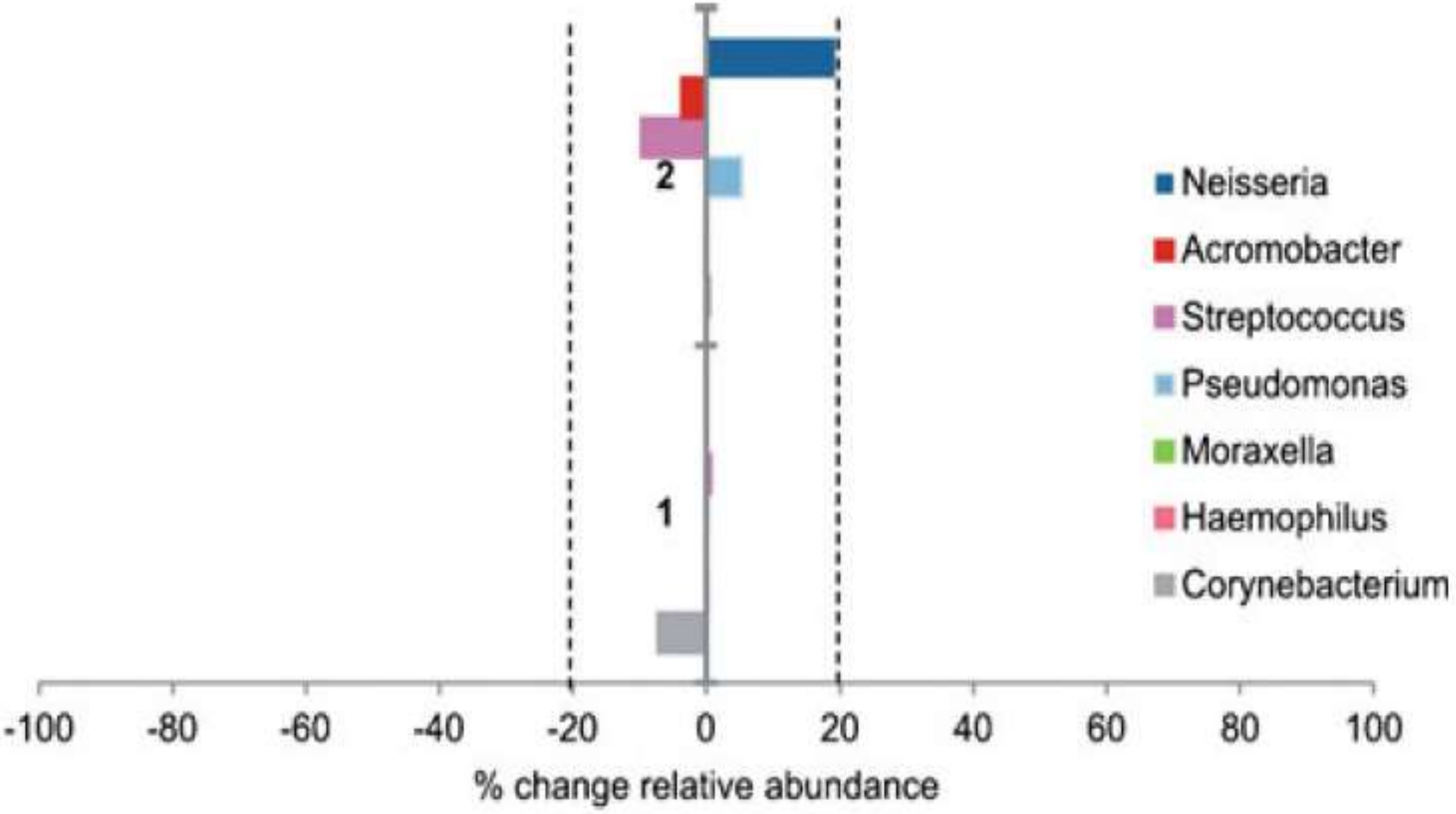




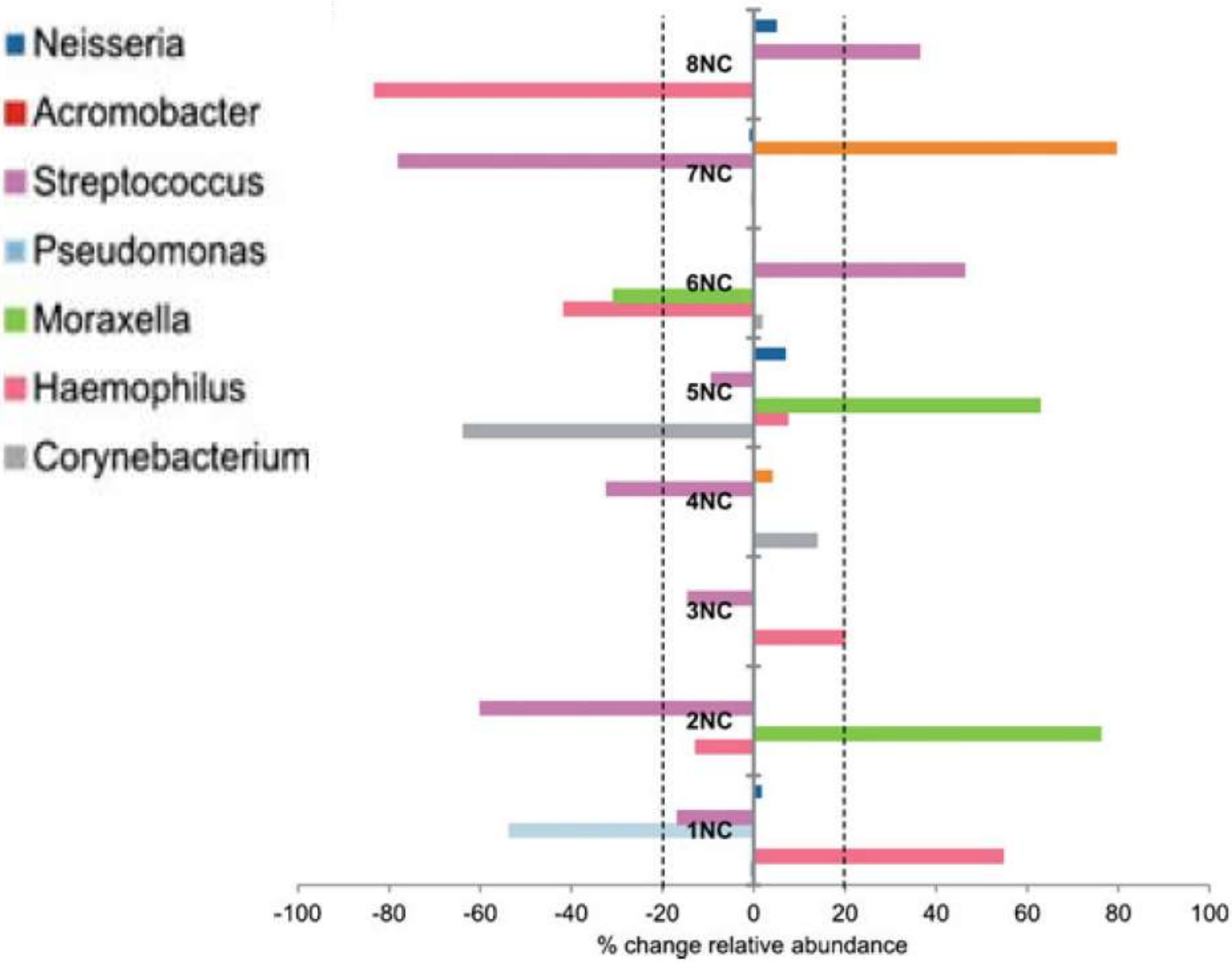
Pseudomonas colonization



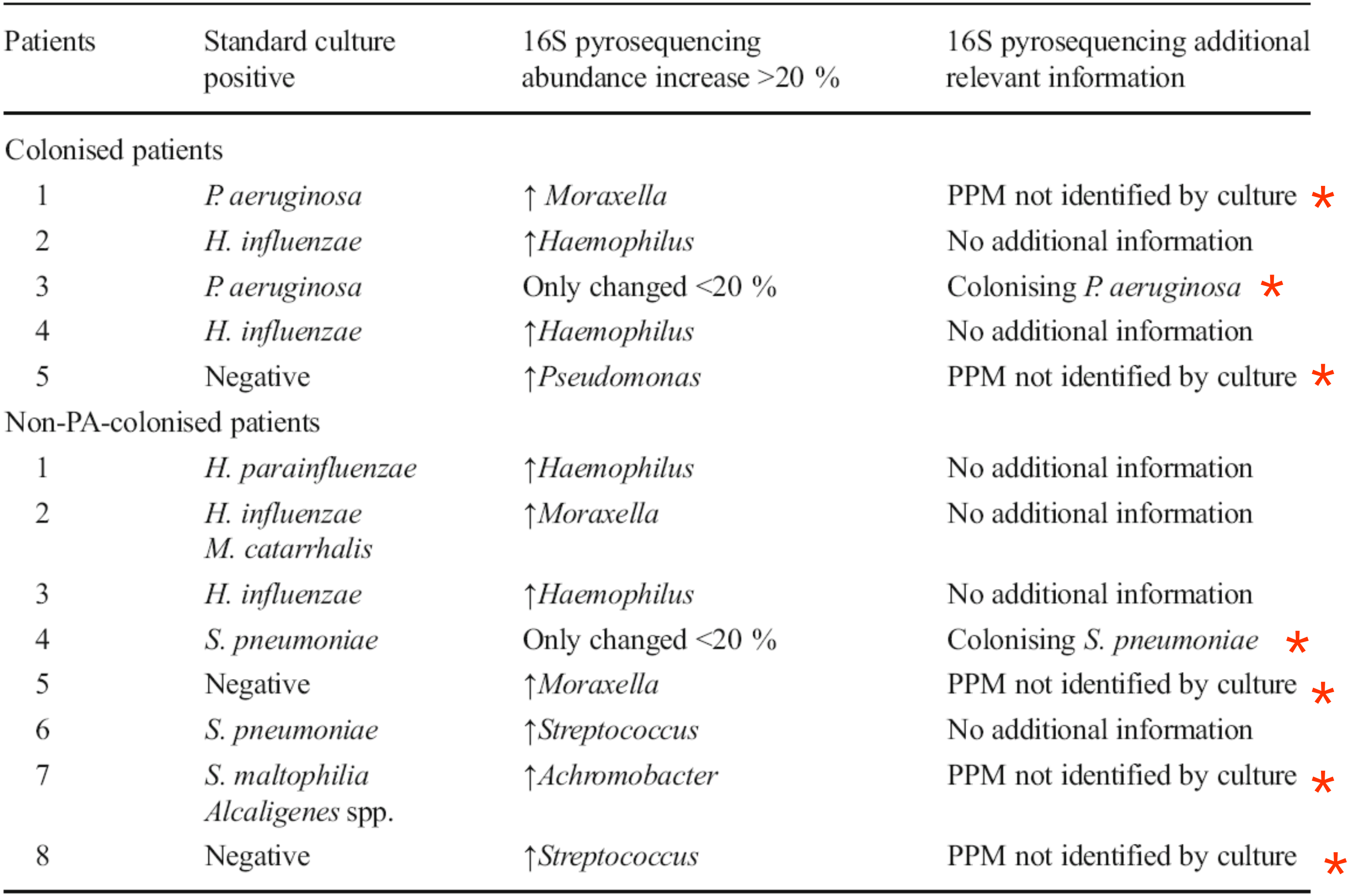
Variability in stability



No *Pseudomonas* colonization



Identifying over culture

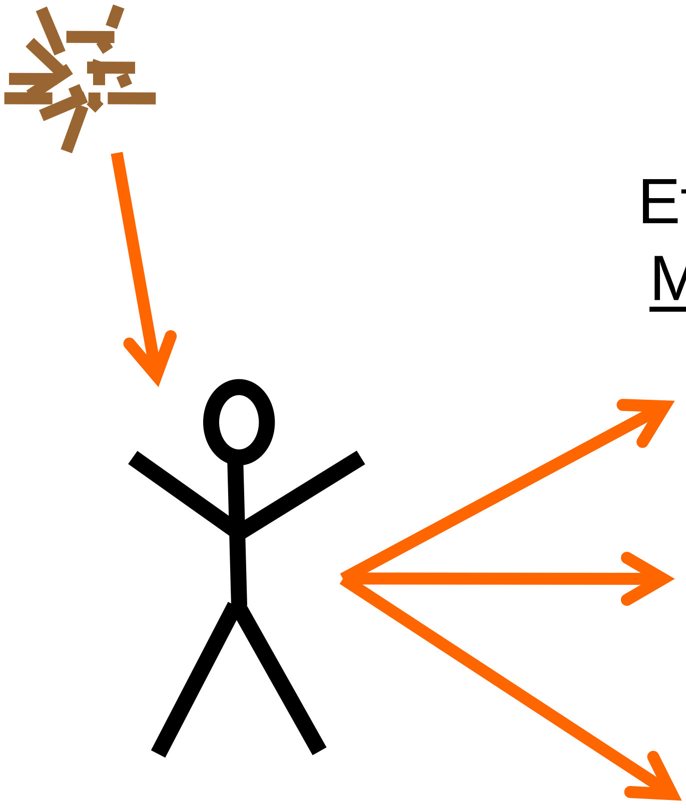


Patients	Standard culture positive	16S pyrosequencing abundance increase >20 %	16S pyrosequencing additional relevant information
Colonised patients			
1	*P. aeruginosa*	↑ *Moraxella*	PPM not identified by culture *
2	*H. influenzae*	↑*Haemophilus*	No additional information
3	*P. aeruginosa*	Only changed <20 %	Colonising *P. aeruginosa* *
4	*H. influenzae*	↑*Haemophilus*	No additional information
5	Negative	↑*Pseudomonas*	PPM not identified by culture *
Non-PA-colonised patients			
1	*H. parainfluenzae*	↑*Haemophilus*	No additional information
2	*H. influenzae* *M. catarrhalis*	↑*Moraxella*	No additional information
3	*H. influenzae*	↑*Haemophilus*	No additional information
4	*S. pneumoniae*	Only changed <20 %	Colonising *S. pneumoniae* *
5	Negative	↑*Moraxella*	PPM not identified by culture *
6	*S. pneumoniae*	↑*Streptococcus*	No additional information
7	*S. maltophilia* *Alcaligenes* spp.	↑*Achromobacter*	PPM not identified by culture *
8	Negative	↑*Streptococcus*	PPM not identified by culture *

Culture-independent microbiology in COPD

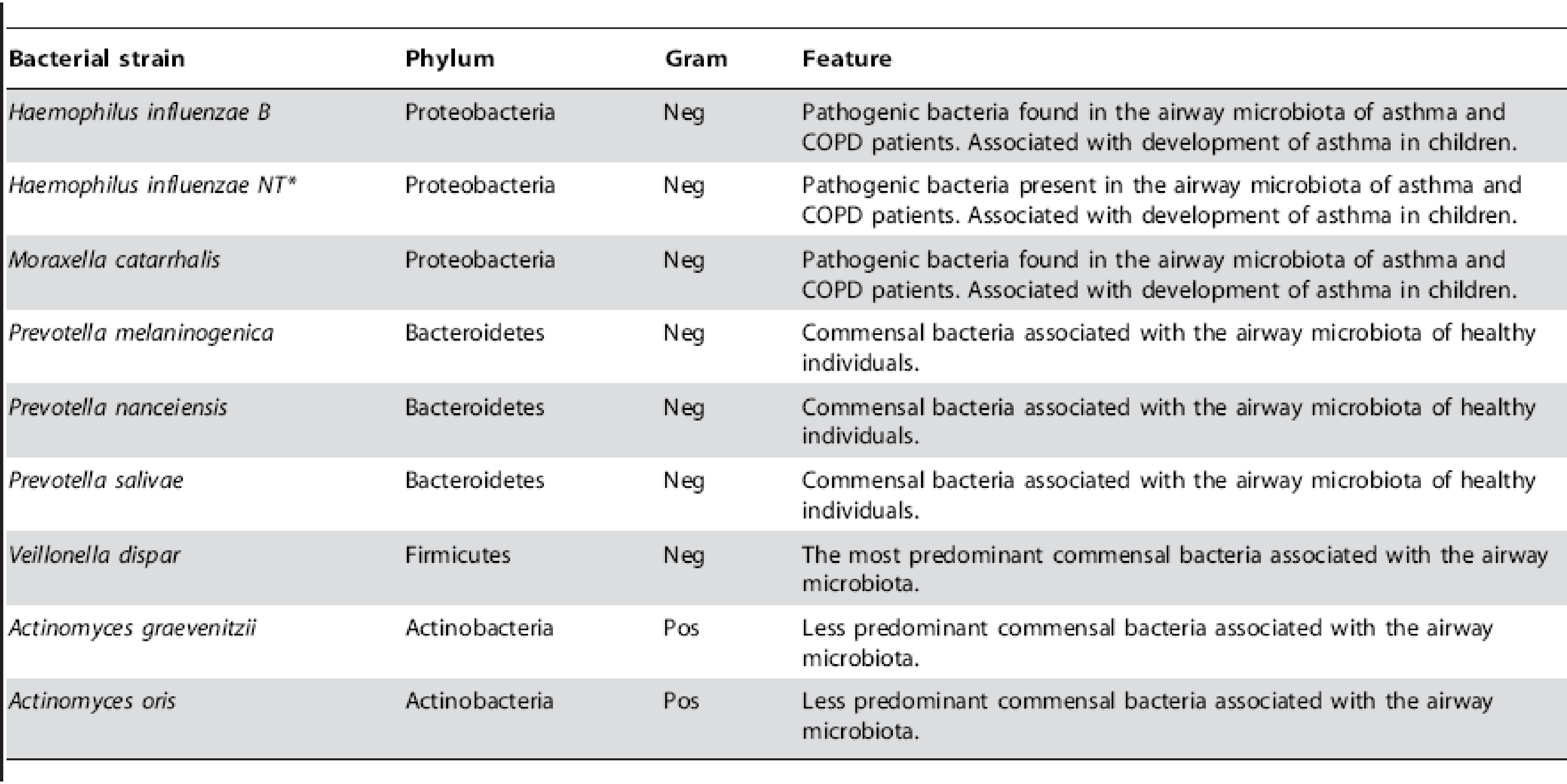
- Identify additional pathogenic bacteria as a cause of exacerbation.
- Common pathogenic bacteria cause exacerbation in patients colonized by *P. aeruginosa.*
- Loss of bacterial diversity in severe COPD.

Symbiosis



Effect on Microbe	Effect on Host	Type of Microbial Symbiont
+	+	Mutualist
+	0	Commensal
+	–	Parasit Pathogen

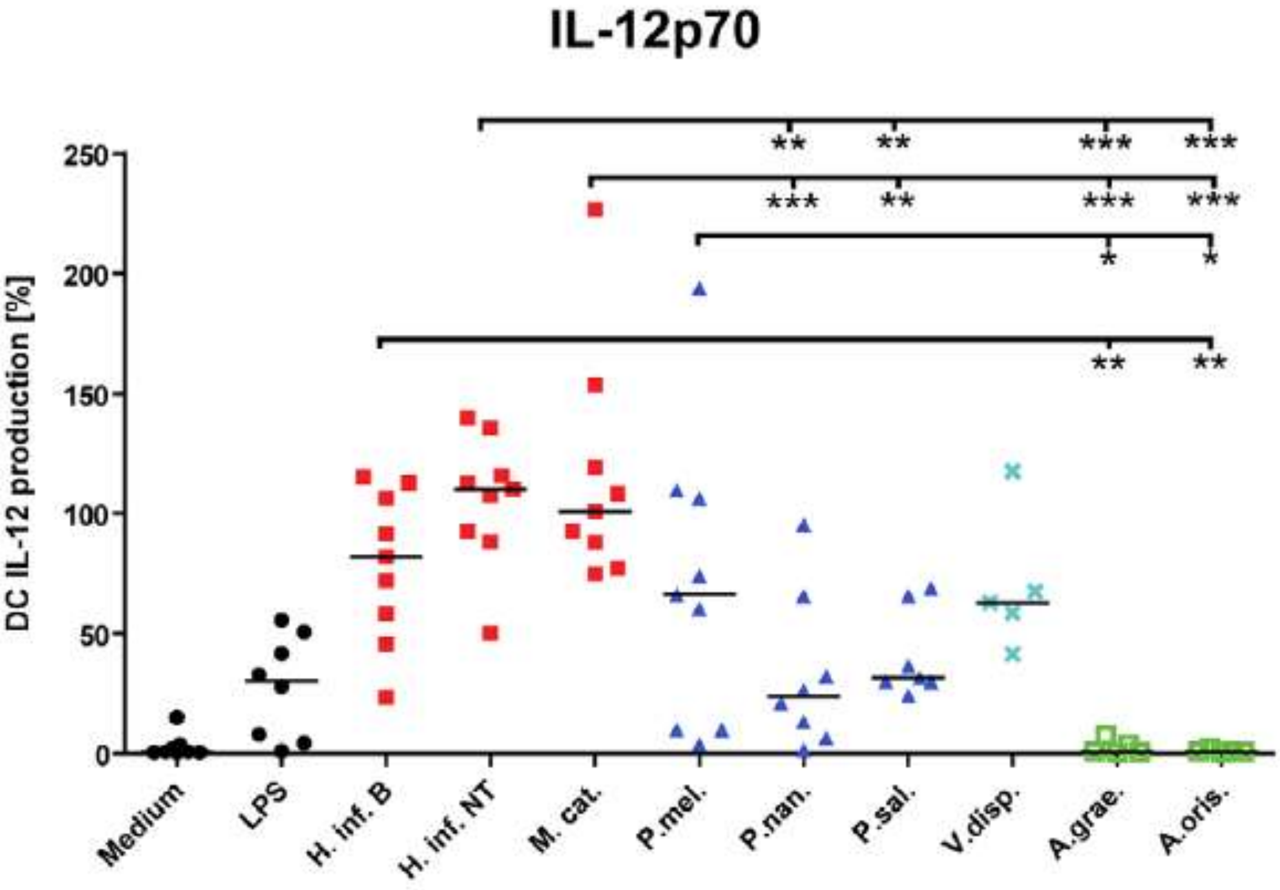
Commensal and pathogens



Bacterial strain	Phylum	Gram	Feature
Haemophilus influenzae B	Proteobacteria	Neg	Pathogenic bacteria found in the airway microbiota of asthma and COPD patients. Associated with development of asthma in children.
*Haemophilus influenzae NT**	Proteobacteria	Neg	Pathogenic bacteria present in the airway microbiota of asthma and COPD patients. Associated with development of asthma in children.
Moraxella catarrhalis	Proteobacteria	Neg	Pathogenic bacteria found in the airway microbiota of asthma and COPD patients. Associated with development of asthma in children.
Prevotella melaninogenica	Bacteroidetes	Neg	Commensal bacteria associated with the airway microbiota of healthy individuals.
Prevotella nanceiensis	Bacteroidetes	Neg	Commensal bacteria associated with the airway microbiota of healthy individuals.
Prevotella salivae	Bacteroidetes	Neg	Commensal bacteria associated with the airway microbiota of healthy individuals.
Veillonella dispar	Firmicutes	Neg	The most predominant commensal bacteria associated with the airway microbiota.
Actinomyces graevenitzii	Actinobacteria	Pos	Less predominant commensal bacteria associated with the airway microbiota.
Actinomyces oris	Actinobacteria	Pos	Less predominant commensal bacteria associated with the airway microbiota.

Larsen. Divergent Pro-Inflammatory Profile of Human Dendritic Cells in Response to Commensal and Pathogenic Bacteria Associated with the Airway Microbiota. PlosOne 2012.

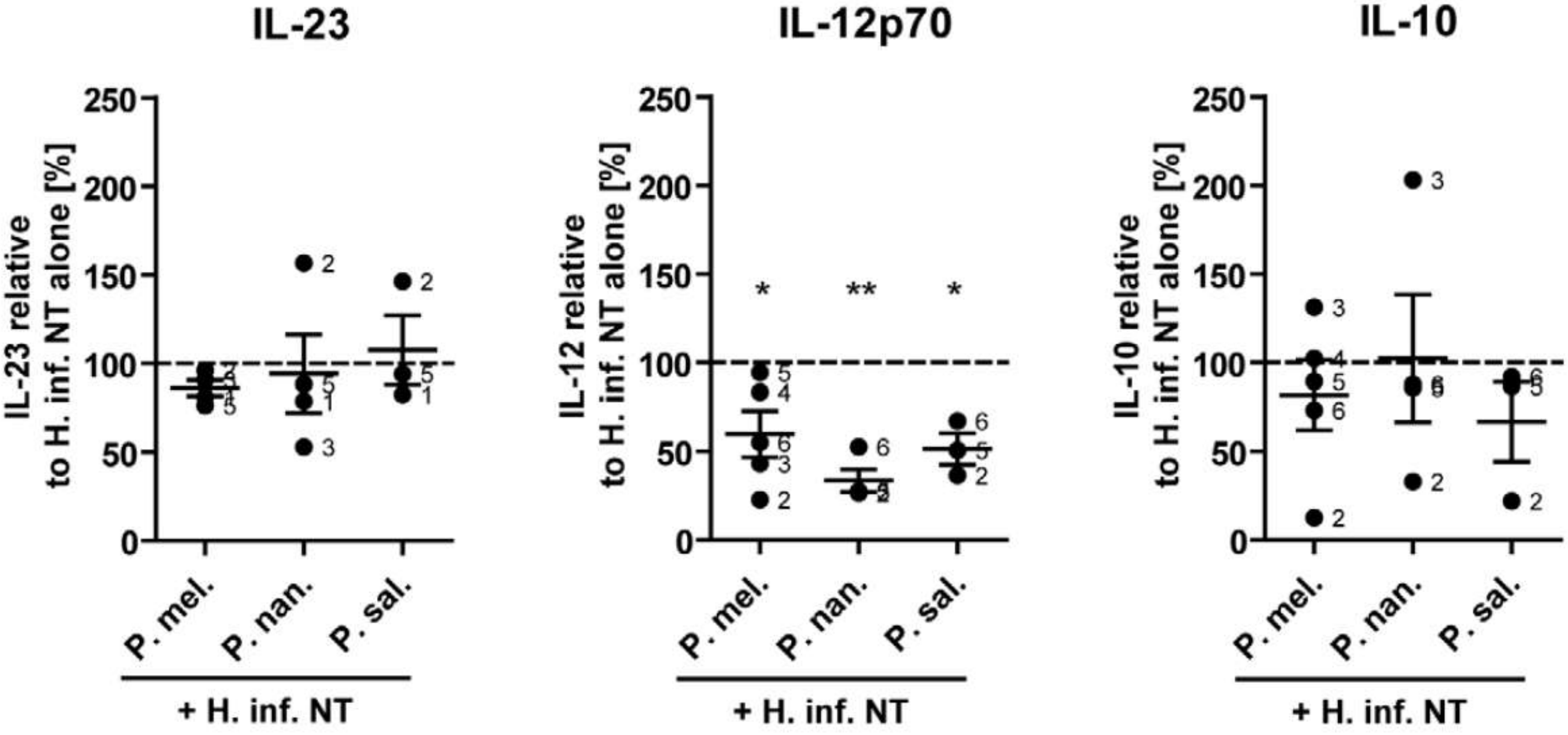
Commensal and pathogens



Larsen. Divergent Pro-Inflammatory Profile of Human Dendritic Cells in Response to Commensal and Pathogenic Bacteria Associated with the Airway Microbiota. PlosOne 2012.

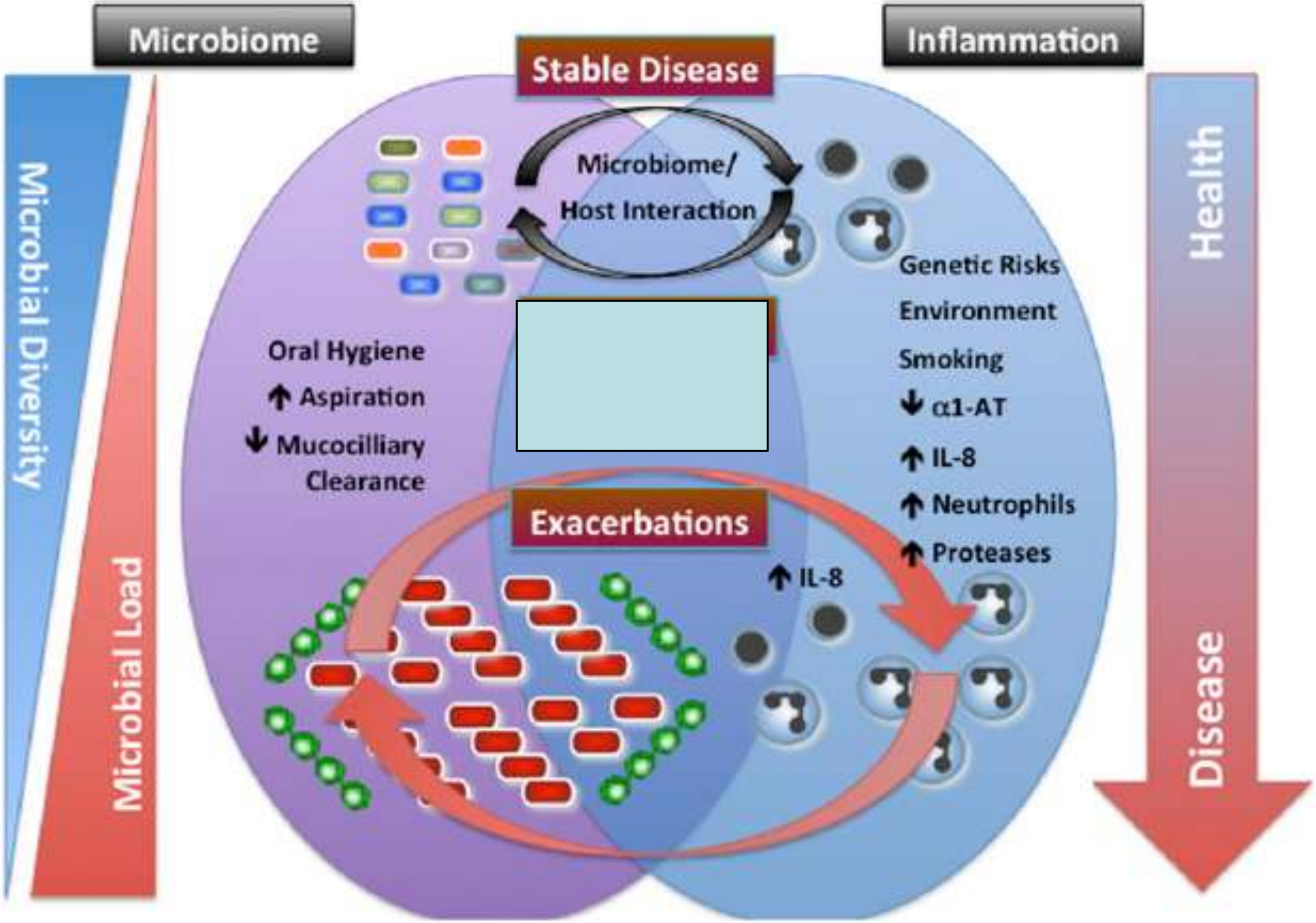
Commensal and pathogens

Modulation of Haemophilus-induced cytokine production in dendritic cells by Prevotella species



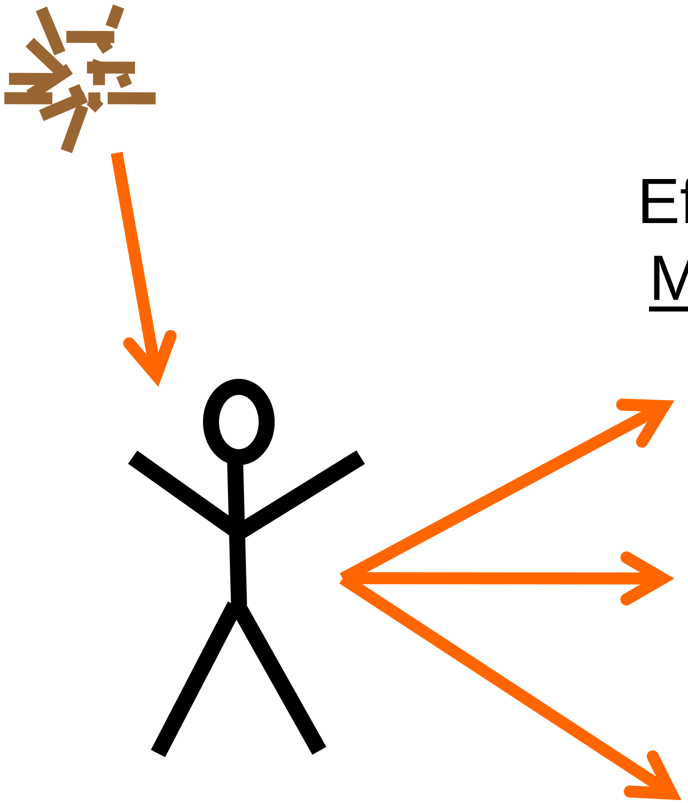
Larsen. Divergent Pro-Inflammatory Profile of Human Dendritic Cells in Response to Commensal and Pathogenic Bacteria Associated with the Airway Microbiota. PlosOne 2012.

Dysbiosis



Segal. Annals ATS 2014

Symbiosis



Effect on Microbe	Effect on Host	Type of Microbial Symbiont
+	+	Mutualist
+	0	Commensal
+	–	Parasit Pathogen