The Promise of Oral Microbiota-Targeted Therapeutics for Oral Diseases

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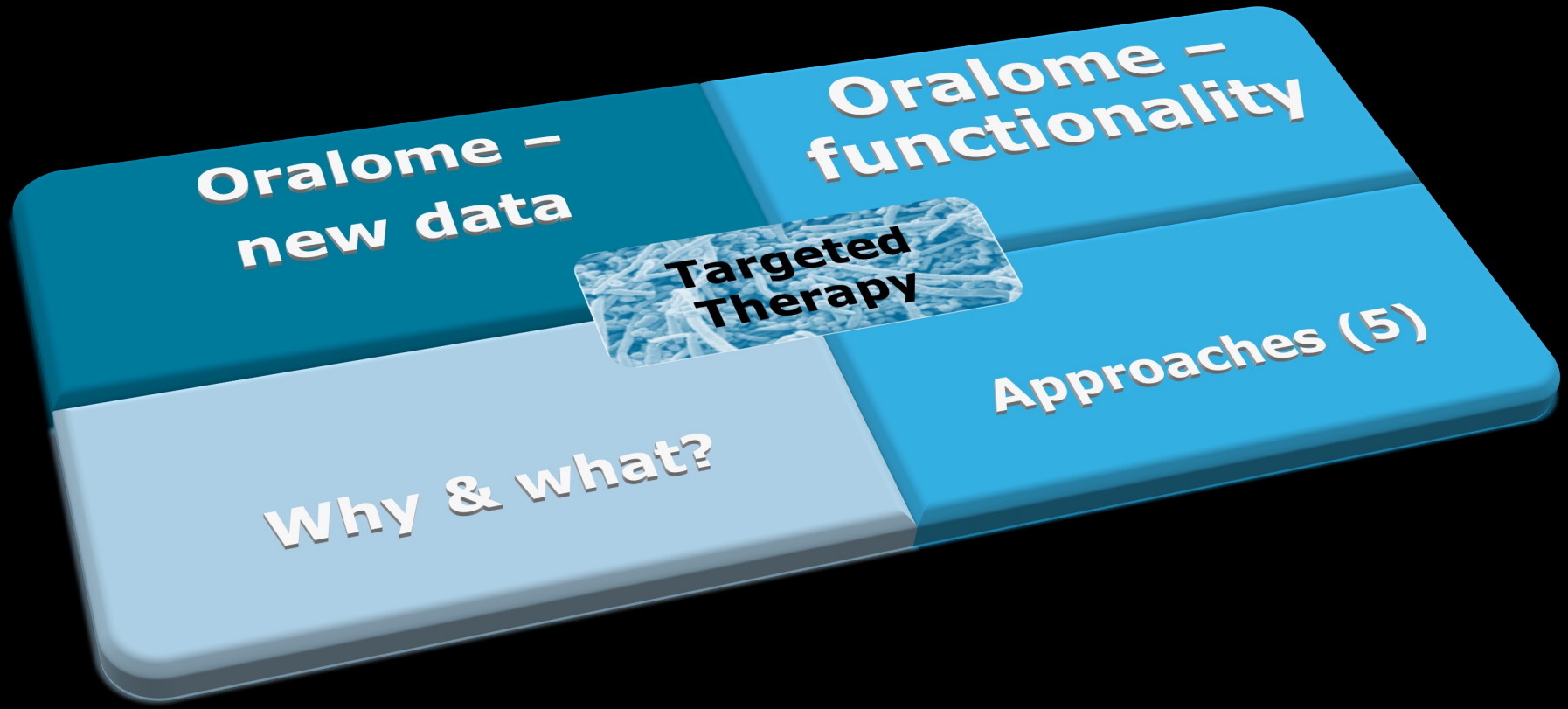
Oralome – new data
Oralome – functionality
Targeted Therapy
Why & what?
Approaches (5)


Oralome –
new data
Targeted
Therapy


Back to Basics


nature
THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
First results from the Human Microbiome Project highlight the healthy variation in our microbial selves
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Structure, function and diversity of the healthy human microbiome

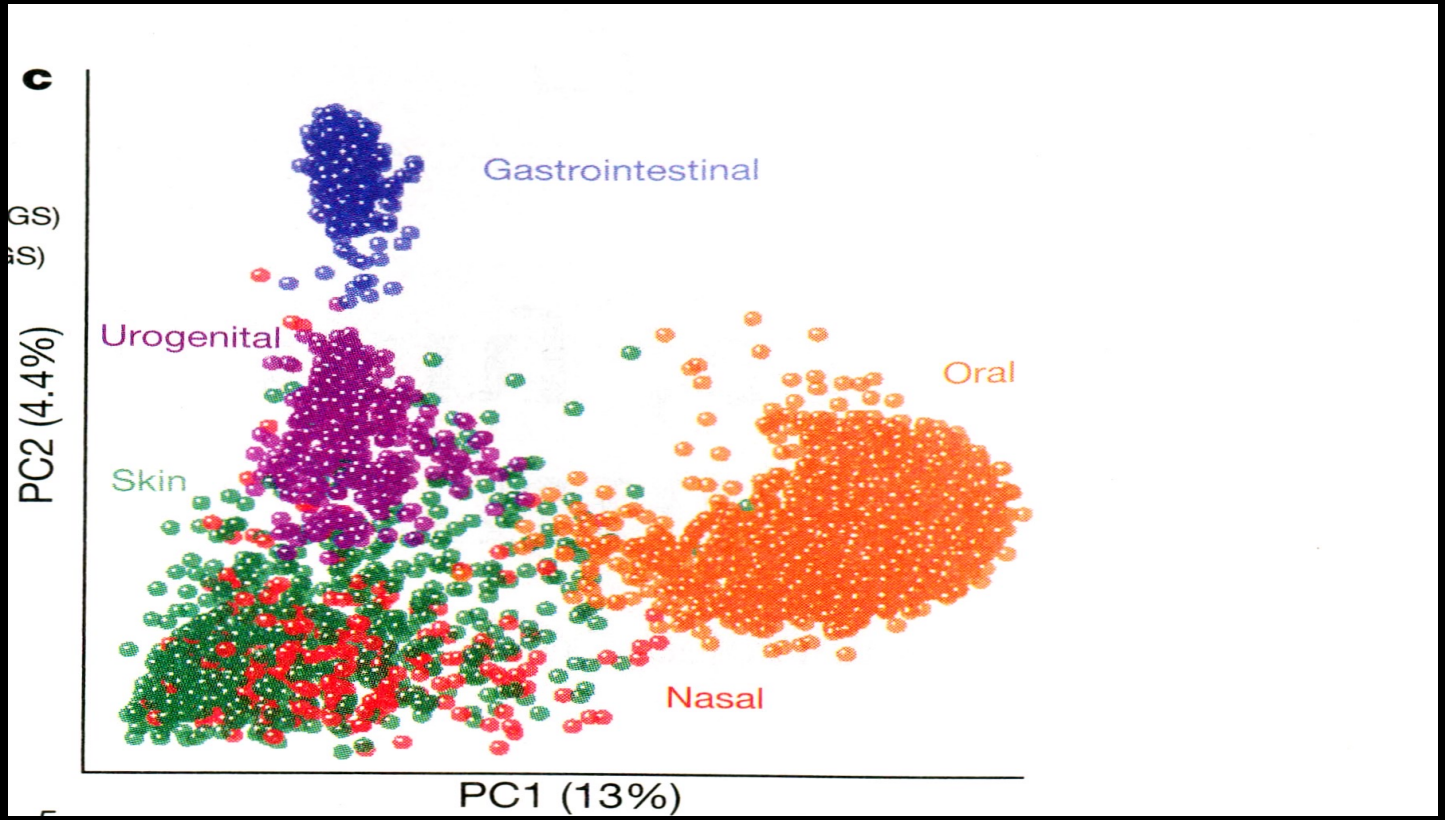
The Human Microbiome Project Consortium*

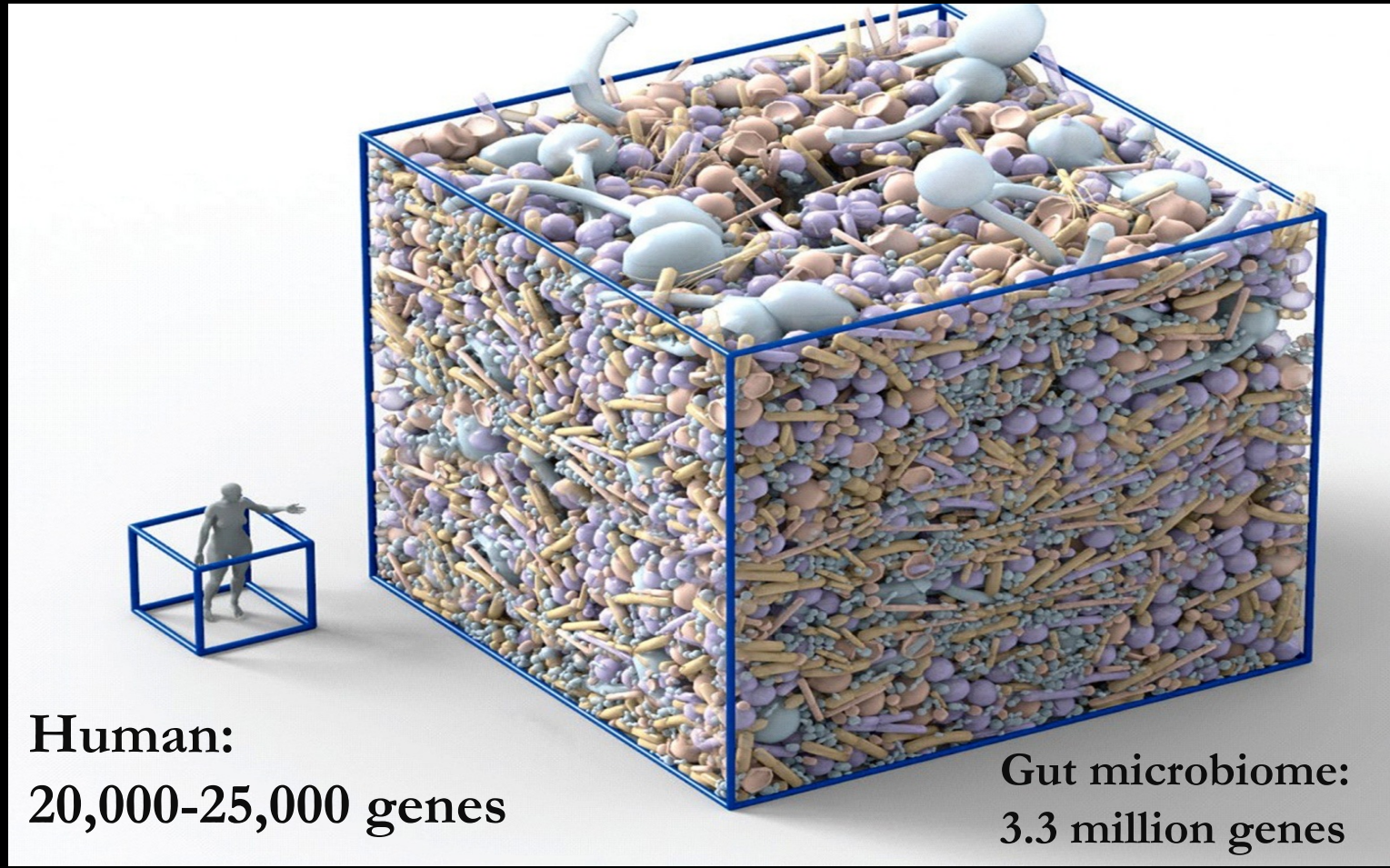
Studies of the human microbiome have revealed that even healthy individuals differ remarkably in the microbes that occupy habitats such as the gut, skin and vagina. Much of this diversity remains unexplained, although diet, environment, host genetics and early microbial exposure have all been implicated. Accordingly, to characterize the ecology of human-associated microbial communities, the Human Microbiome Project has analysed the largest cohort and set of distinct, clinically relevant body habitats so far. We found the diversity and abundance of each habitat's signature microbes to vary widely even among healthy subjects, with strong niche specialization both within and among individuals. The project encountered an estimated 81–99% of the genera, enzyme families and community configurations occupied by the healthy Western microbiome. Metagenomic carriage of metabolic pathways was stable among individuals despite variation in community structure, and ethnic/racial background proved to be one of the strongest associations of both pathways and microbes with clinical metadata. These results thus delineate the range of structural and functional configurations normal in the microbial communities of a healthy population, enabling future characterization of the epidemiology, ecology and translational applications of the human microbiome.

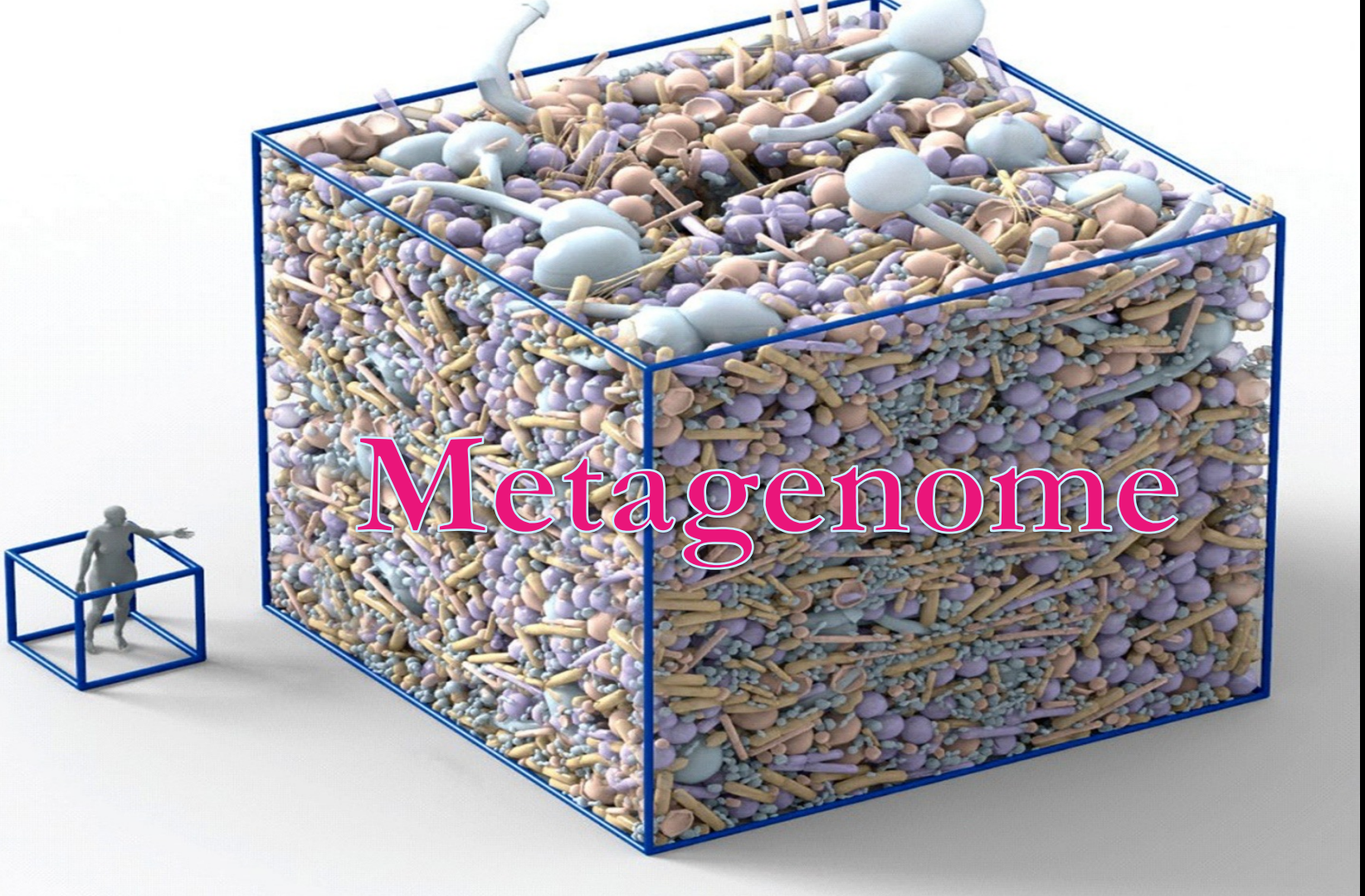
A total of 4,788 specimens from 242 screened and phenotyped adults[1] (129 males, 113 females) were available for this study, representing the majority of the target Human Microbiome Project (HMP) cohort of 300 individuals. Adult subjects lacking evidence of disease were recruited based on a lengthy list of exclusion criteria; we will refer to them here as 'healthy', as defined by the consortium clinical sampling criteria (K. Aagaard *et al.*, manuscript submitted). Women were sampled at 18 body habitats, men at 15 (excluding three vaginal sites), distributed among five major body areas. Nine specimens were collected from the oral cavity and oropharynx: saliva; buccal

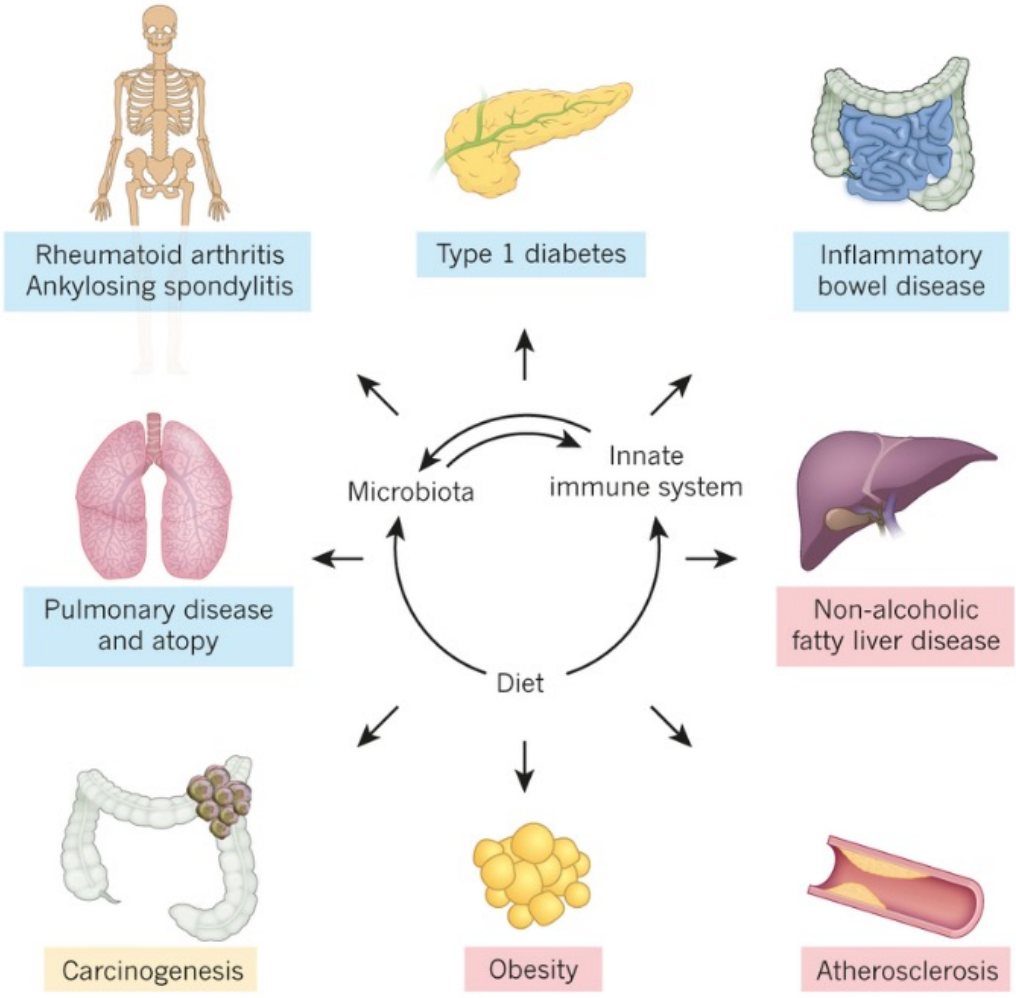
involving microbiome samples collected from healthy volunteers at two distinct geographic locations in the United States, we have defined the microbial communities at each body habitat, encountering 81–99% of predicted genera and saturating the range of overall community configurations (Fig. 1, Supplementary Fig. 1 and Supplementary Table 1; see also Fig. 4). Oral and stool communities were especially diverse in terms of community membership, expanding prior observations[5], and vaginal sites harboured particularly simple communities (Fig. 1a). This study established that these patterns of alpha diversity (within samples) differed markedly from comparisons between

Diversity of the Oral Microbiome is Unique to individuals

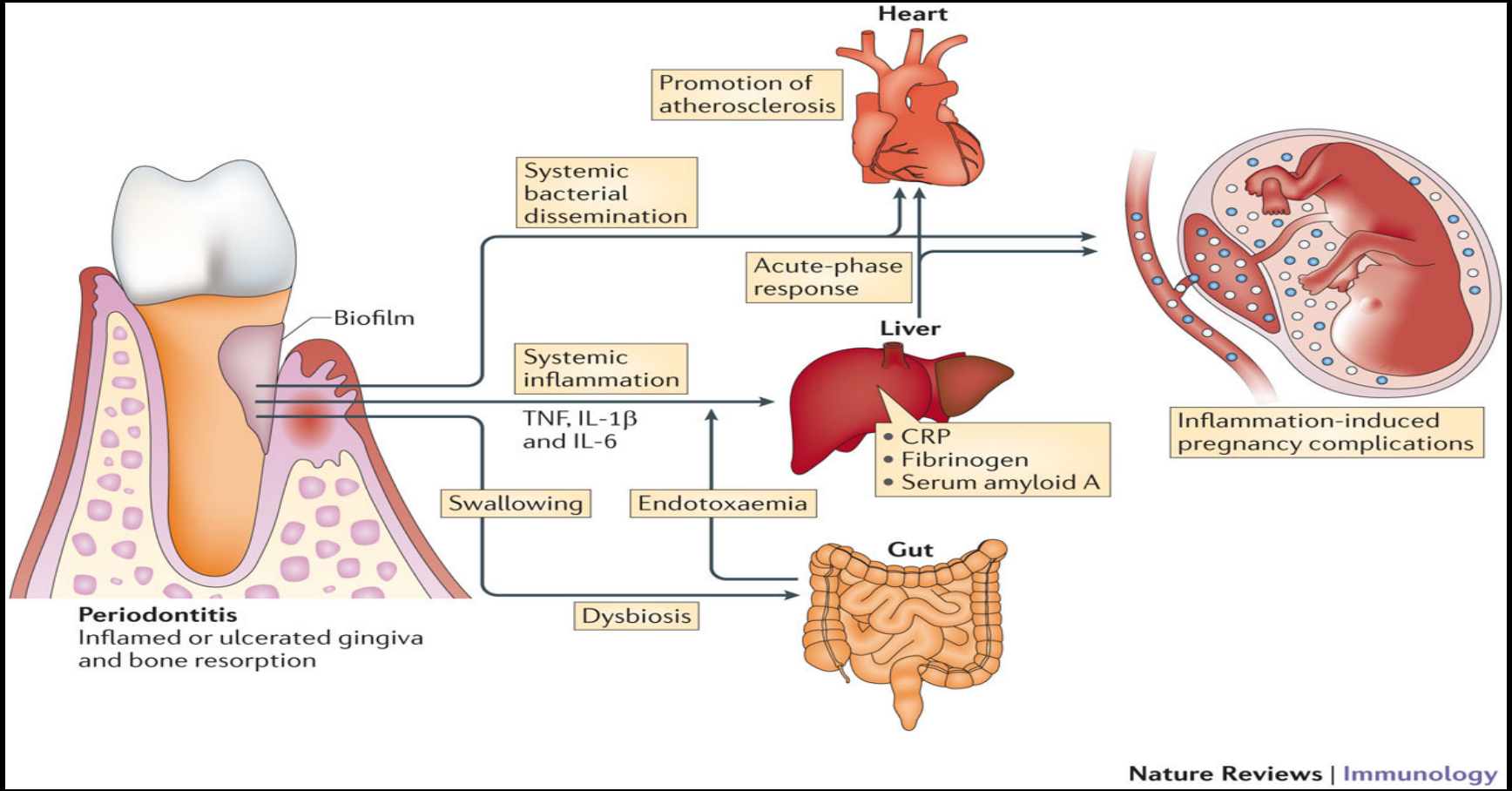


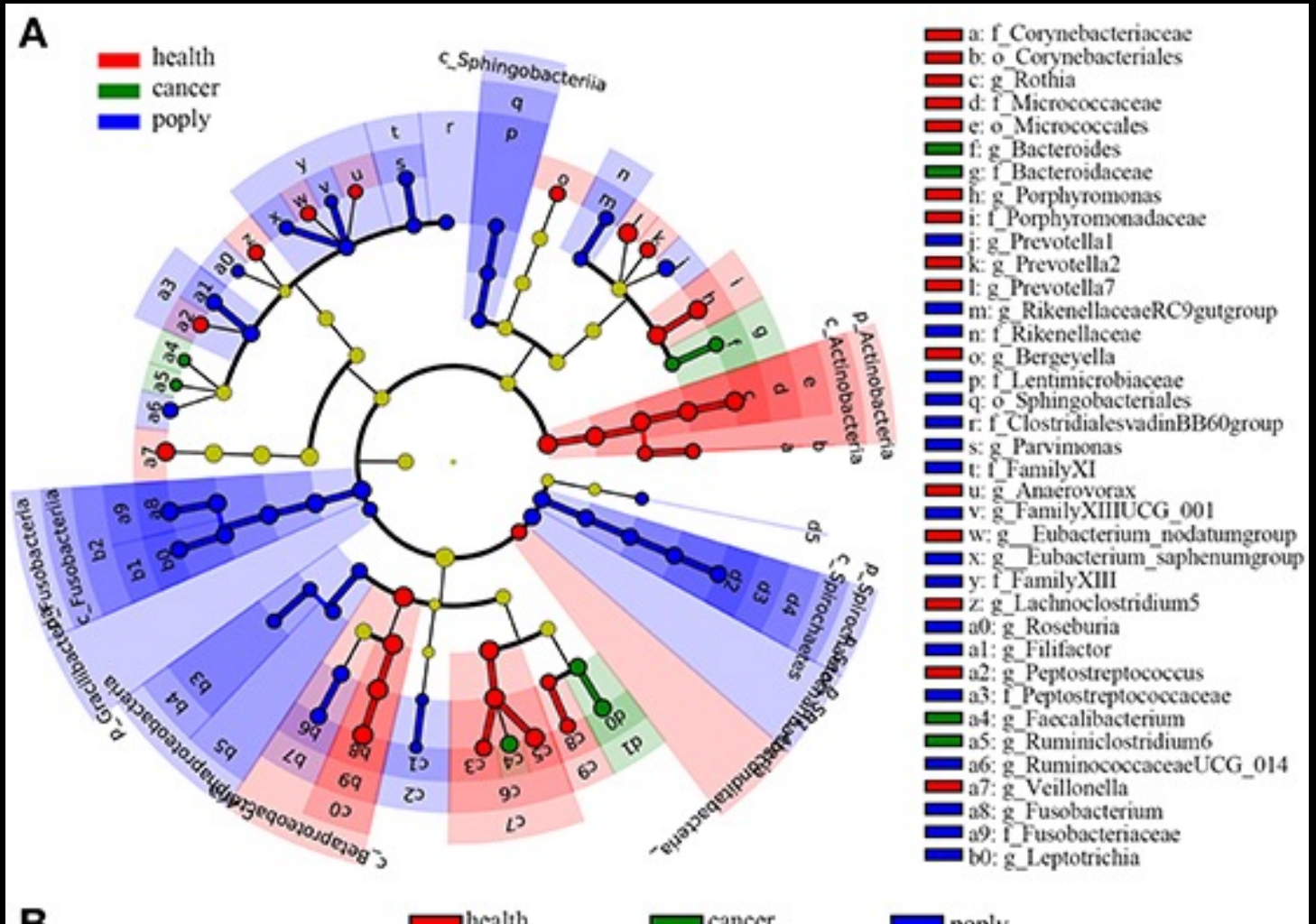

Human:
20,000-25,000 genes
Gut microbiome:
3.3 million genes

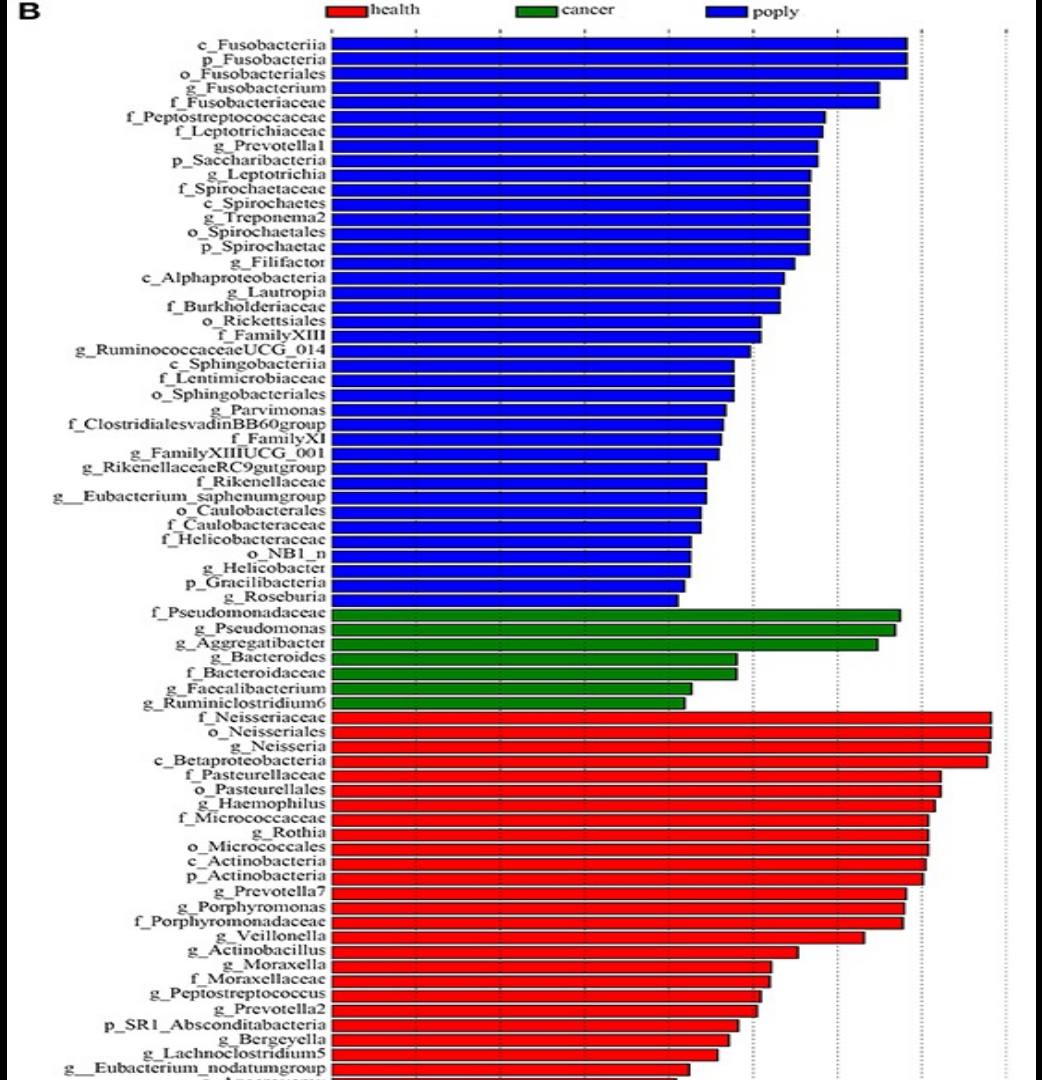

Metagenome

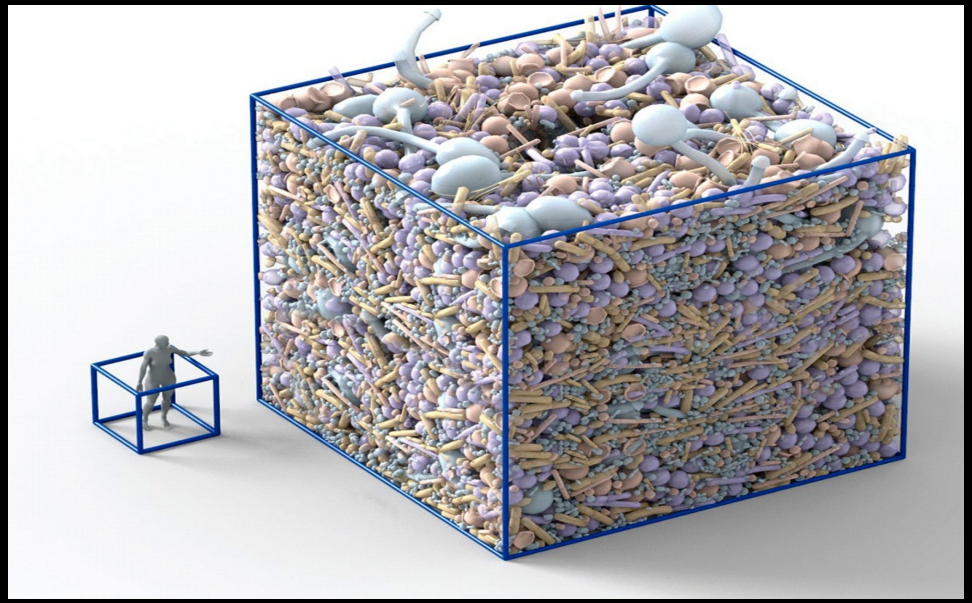

Rheumatoid arthritis
Ankylosing spondylitis
Type 1 diabetes
Inflammatory bowel disease
Microbiota
Innate immune system
Pulmonary disease and atopy
Non-alcoholic fatty liver disease
Diet
Carcinogenesis
Obesity
Atherosclerosis

Oral- Systemic Axis




A
health
cancer
poply
c_Sphingobacteria
p_Actinobacteria
c_Actinobacteria
p_Spirochaetes
c_Spirochaetes
p_Bacteroidetes
c_Betaproteobacteria
p_Gracilibacteria
c_Fusobacteriia
a: f_Corynebacteriaceae
b: o_Corynebacteriales
c: g_Rothia
d: f_Micrococcaceae
e: o_Micrococcales
f: g_Bacteroides
g: f_Bacteroidaceae
h: g_Porphyromonas
i: f_Porphyromonadaceae
j: g_Prevotella1
k: g_Prevotella2
l: g_Prevotella7
m: g_RikenellaceaeRC9gutgroup
n: f_Rikenellaceae
o: g_Bergeyella
p: f_Lentimicrobiaceae
q: o_Sphingobacteriales
r: f_ClostridialesvadinBB60group
s: g_Parvimonas
t: f_FamilyXI
u: g_Anaerovorax
v: g_FamilyXIIIUCG_001
w: g__Eubacterium_nodatumgroup
x: g__Eubacterium_saphenumgroup
y: f_FamilyXIII
z: g_Lachnoclostridium5
a0: g_Roseburia
a1: g_Filifactor
a2: g_Peptostreptococcus
a3: f_Peptostreptococcaceae
a4: g_Faecalibacterium
a5: g_Ruminiclostridium6
a6: g_RuminococcaceaeUCG_014
a7: g_Veillonella
a8: g_Fusobacterium
a9: f_Fusobacteriaceae
b0: g_Leptotrichia
B
health
cancer
poply


B
health
cancer
poply
c_Fusobacteriia
p_Fusobacteria
o_Fusobacteriales
g_Fusobacterium
f_Fusobacteriaceae
f_Peptostreptococcaceae
f_Leptotrichiaceae
g_Prevotella1
p_Saccharibacteria
g_Leptotrichia
f_Spirochaetaceae
c_Spirochaetes
g_Treponema2
o_Spirochaetales
p_Spirochaetae
g_Filifactor
c_Alphaproteobacteria
g_Lautropia
f_Burkholderiaceae
o_Rickettsiales
f_FamilyXIII
g_RuminococcaceaeUCG_014
c_Sphingobacteriia
f_Lentimicrobiaceae
o_Sphingobacteriales
g_Parvimonas
f_ClostridialesvadinBB60group
f_FamilyXI
g_FamilyXIIIUCG_001
g_RikenellaceaeRC9gutgroup
f_Rikenellaceae
g__Eubacterium_saphenumgroup
o_Caulobacterales
f_Caulobacteraceae
f_Helicobacteraceae
o_NB1_n
g_Helicobacter
p_Gracilibacteria
g_Roseburia
f_Pseudomonadaceae
g_Pseudomonas
g_Aggregatibacter
g_Bacteroides
f_Bacteroidaceae
g_Faecalibacterium
g_Ruminiclostridium6
f_Neisseriaceae
o_Neisseriales
g_Neisseria
c_Betaproteobacteria
f_Pasteurellaceae
o_Pasteurellales
g_Haemophilus
f_Micrococcaceae
g_Rothia
o_Micrococcales
c_Actinobacteria
p_Actinobacteria
g_Prevotella7
g_Porphyromonas
f_Porphyromonadaceae
g_Veillonella
g_Actinobacillus
g_Moraxella
f_Moraxellaceae
g_Peptostreptococcus
g_Prevotella2
p_SR1_Absconditabacteria
g_Bergeyella
g_Lachnoclostridium5
g__Eubacterium_nodatumgroup



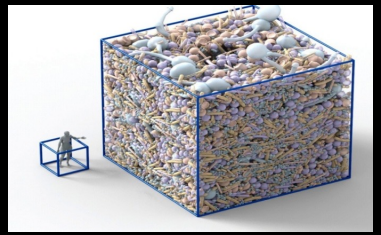
- Human Microbiome
- Oral Microbiome

Simple Definition: Oral Microbiome

`The totality of all the organisms in the human oral cavity and their ecosystem`

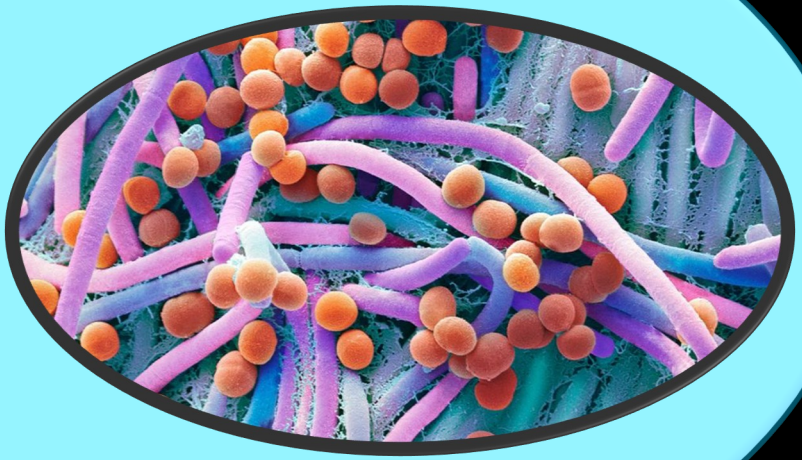
Oral Microbiota

`The totality of bacteria, archea, fungi, viruses, phages and protozoa in the human oral cavity`

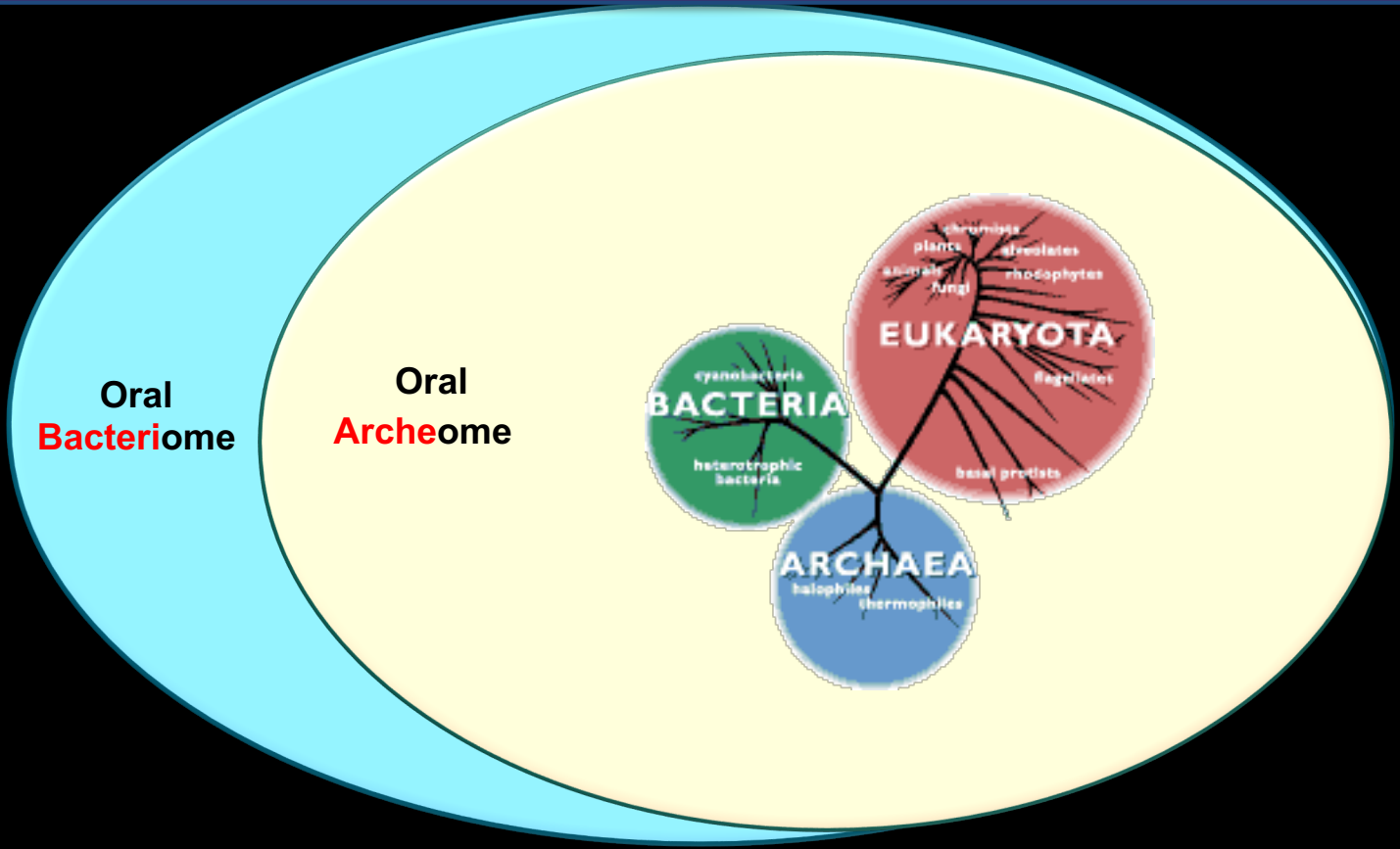


Oral Microbiome

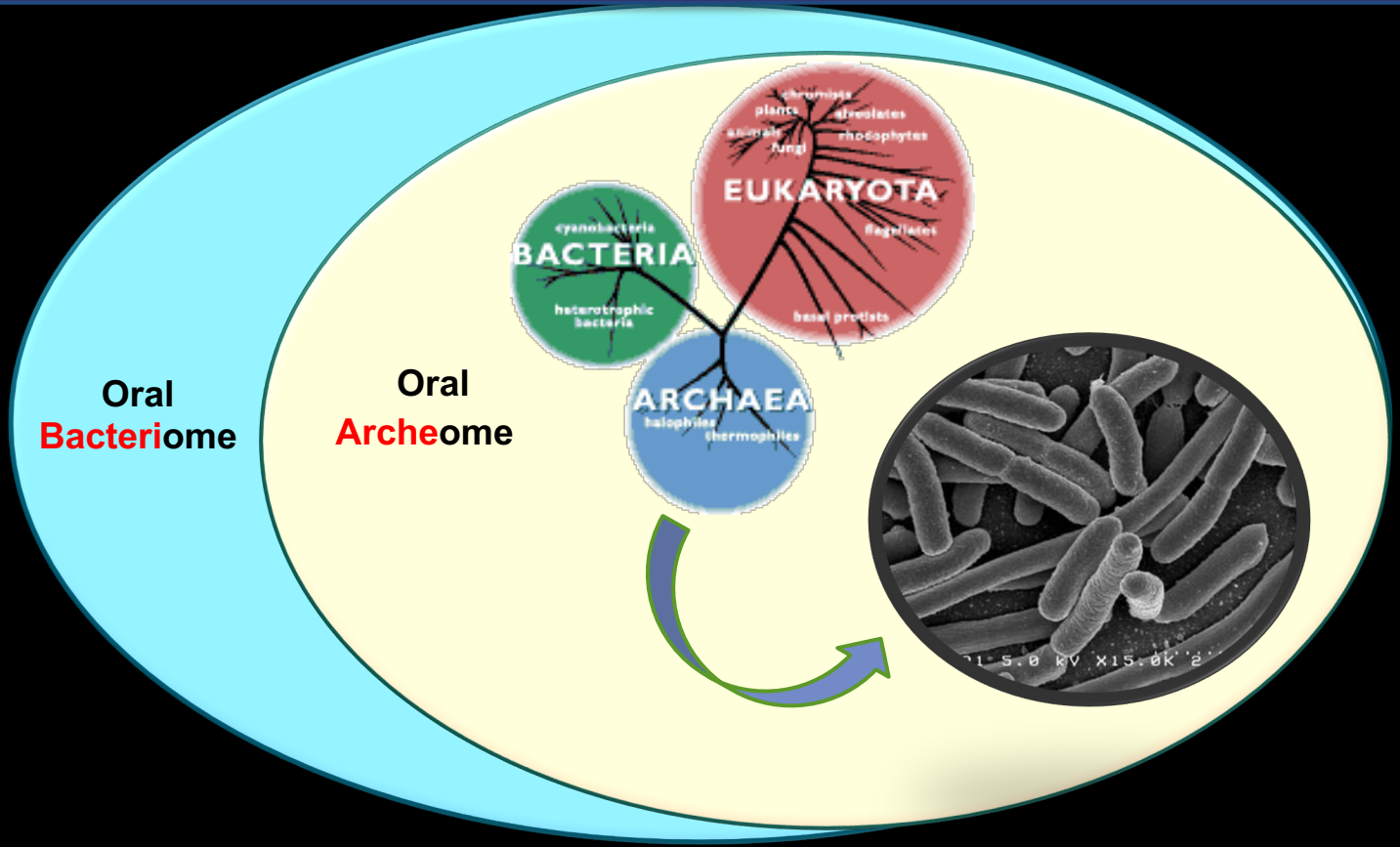
Oral
Bacteriome



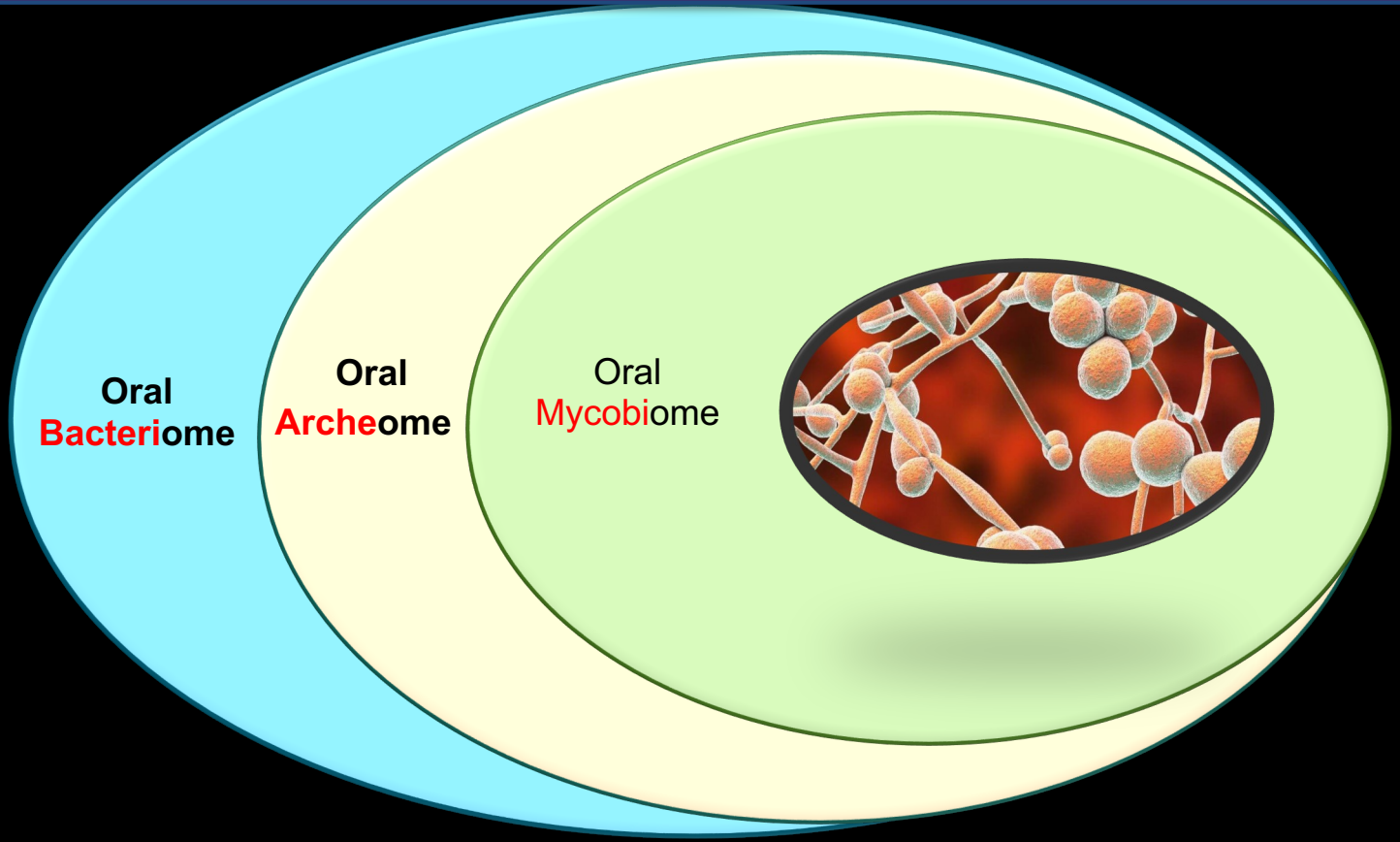
Oral Mircobiome

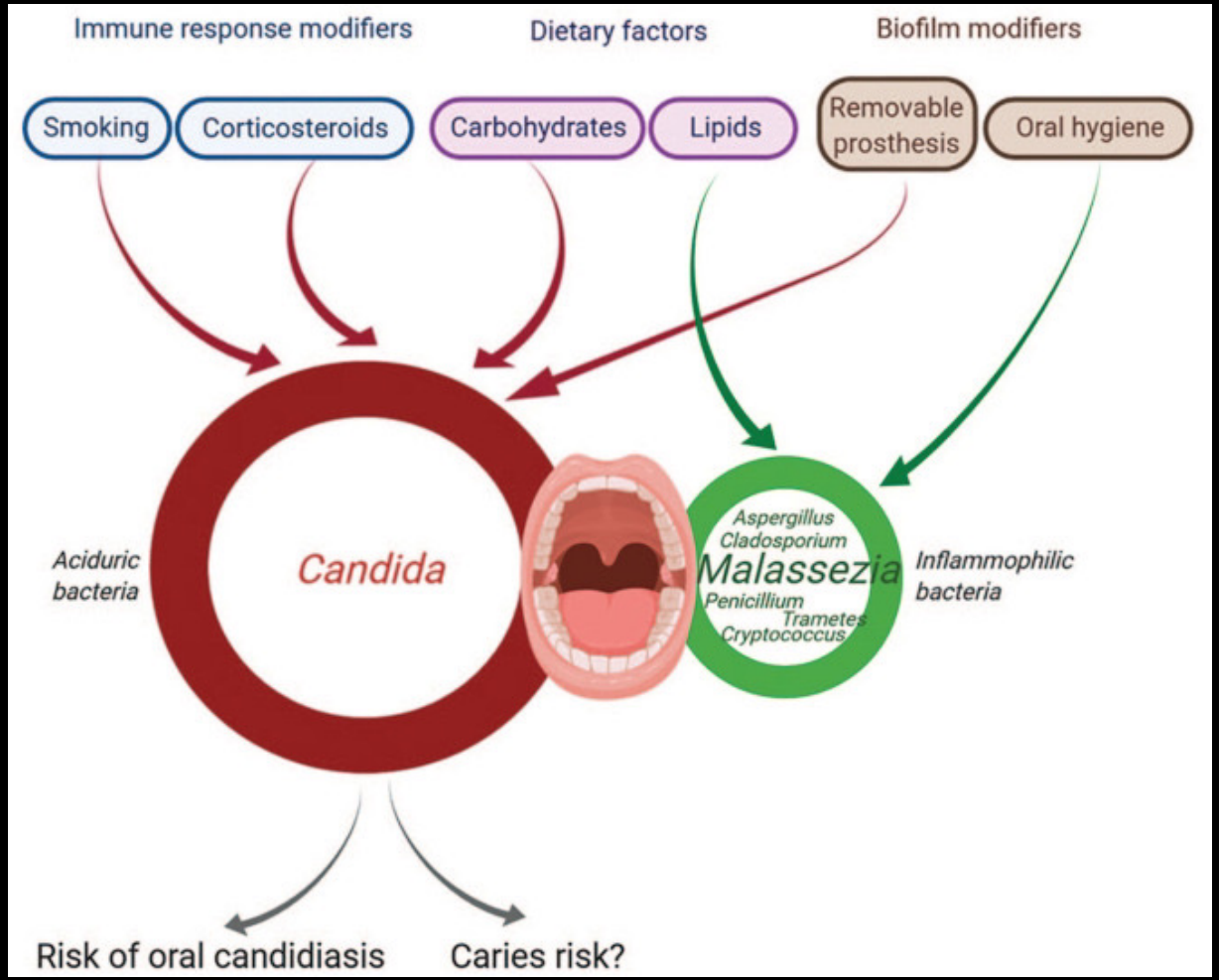


Oral Mircobiome

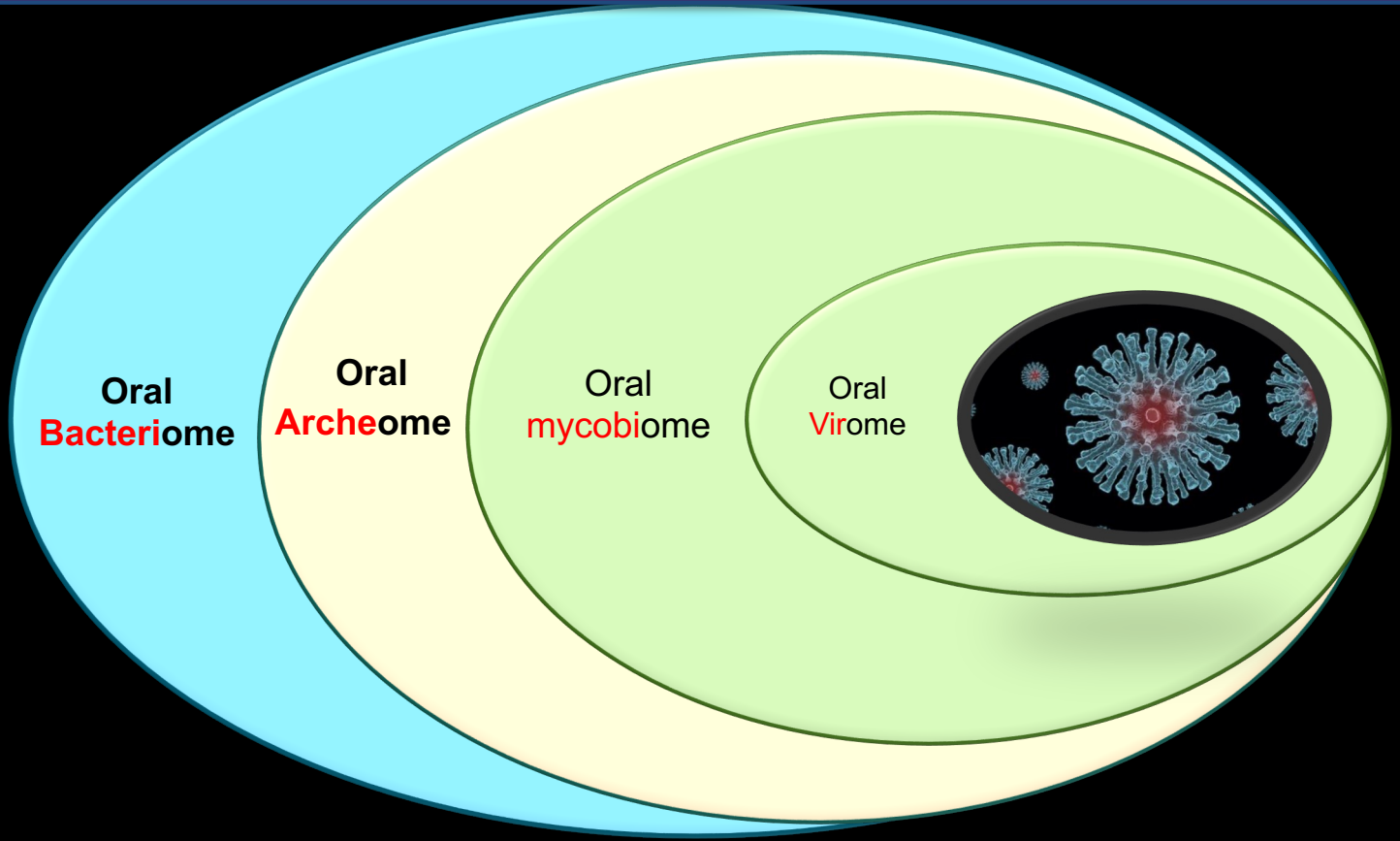


Oral Microbiome



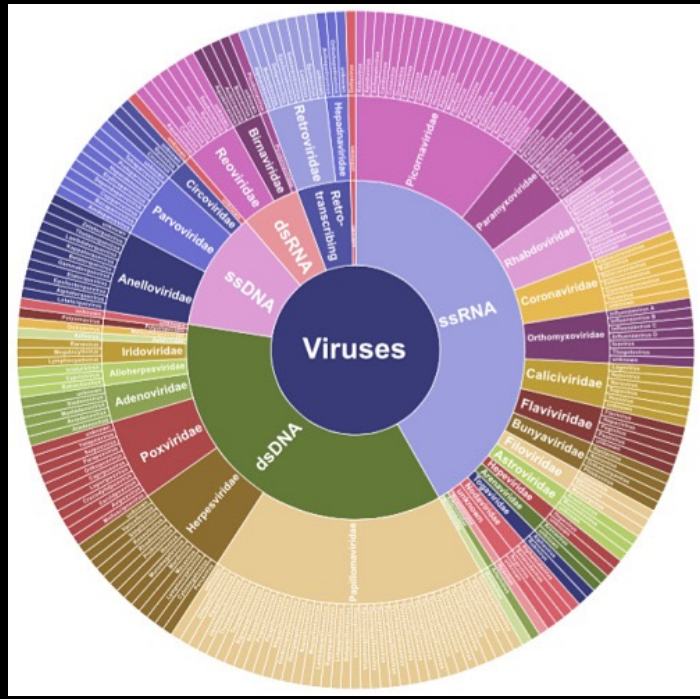

Immune response modifiers
Dietary factors
Biofilm modifiers
Smoking
Corticosteroids
Carbohydrates
Lipids
Removable prosthesis
Oral hygiene
Aciduric bacteria
Candida
Aspergillus
Cladosporium
Malassezia
Penicillium
Trametes
Cryptococcus
Inflammophilic bacteria
Risk of oral candidiasis
Caries risk?

Oral Microbiome



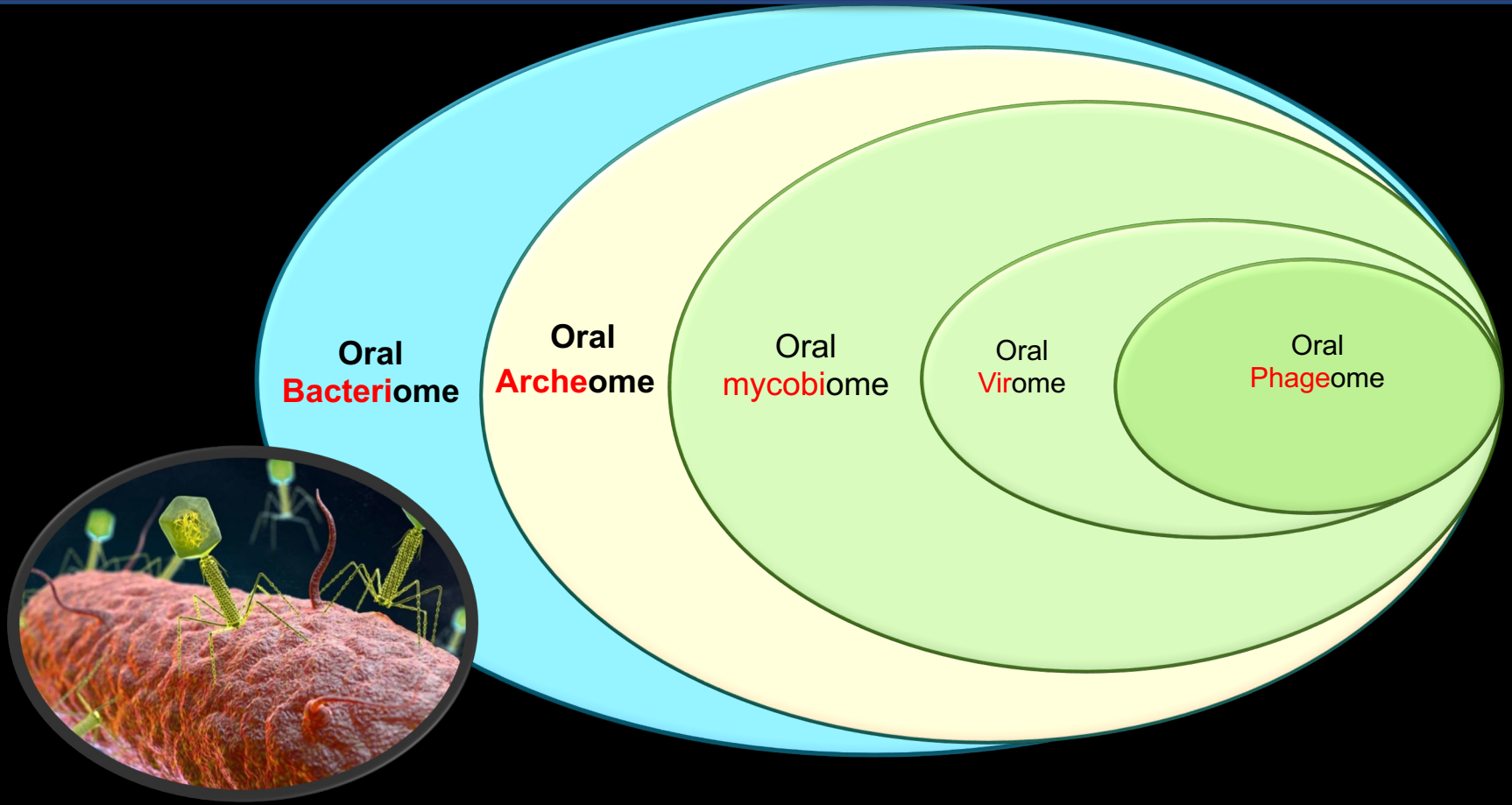
Oral Virome

- Members of the human herpesvirus and human papillomavirus families cause the most common primary viral infections of the oral cavity
- Herpes viruses
  - Alpha
  - Beta
  - gamma

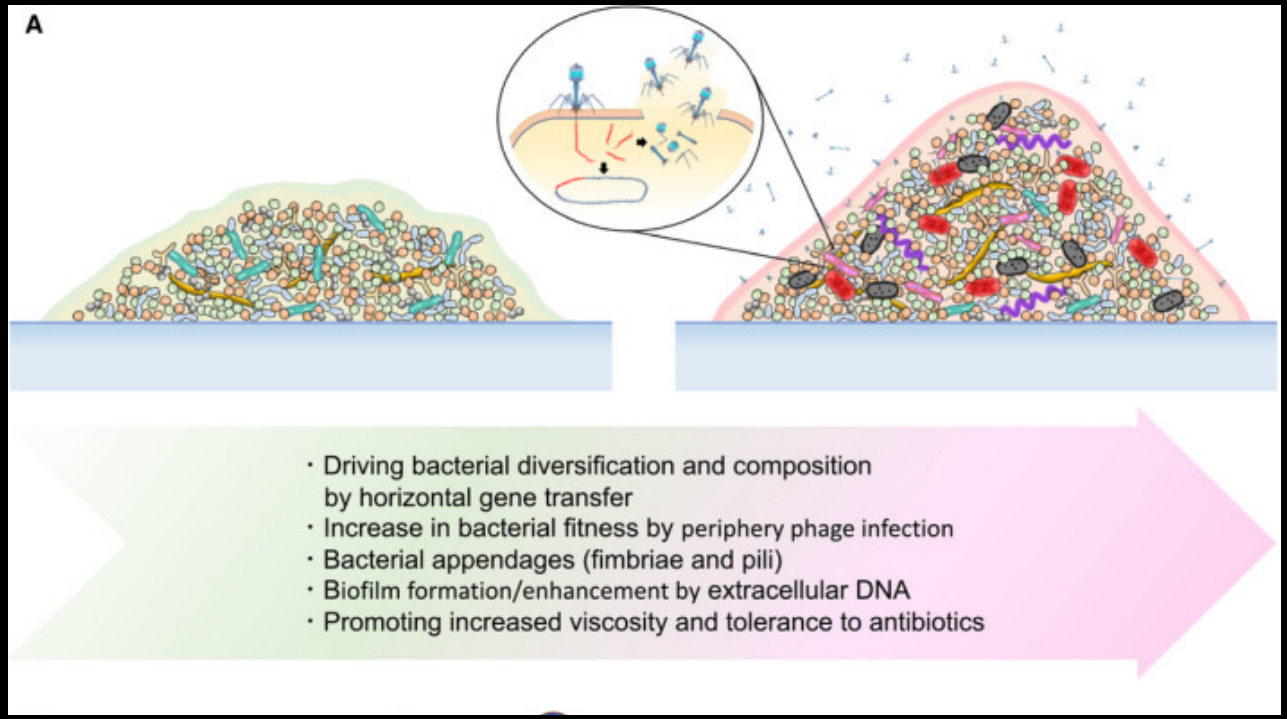


Martínez A, Kuraji R, Kapila YL. The human oral virome: Shedding light on the dark matter. Periodontol 2000.
2021 Oct;87(1):282-298. doi: 10.1111/prd.12396.
PMID: 34463988; PMCID: PMC8457075.

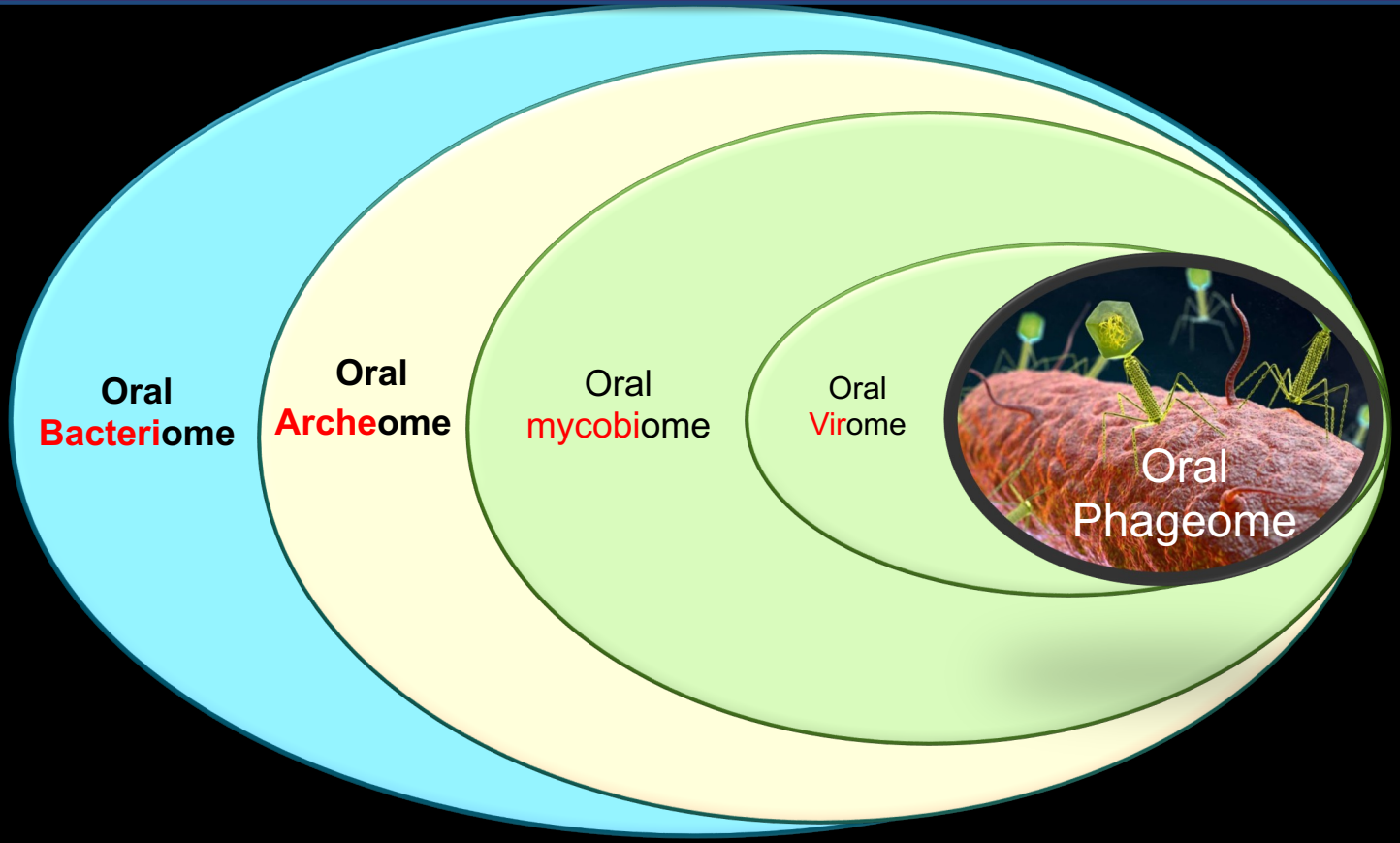
Oral Microbiome



Phageome of the Oral Cavity



Oral Microbiome



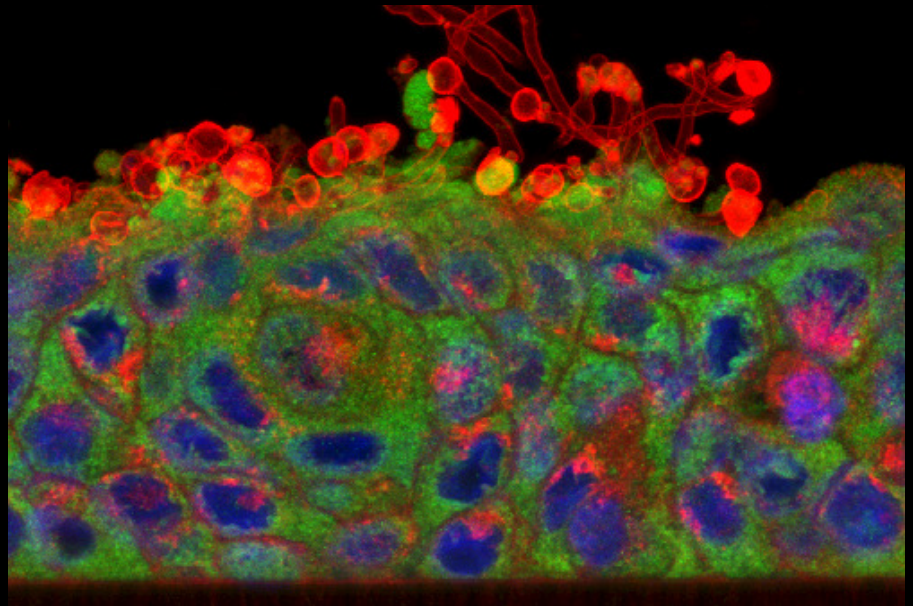
Note: Oral protozoome not included


Oralome – new data
Oralome – functionality
Targeted Therapy

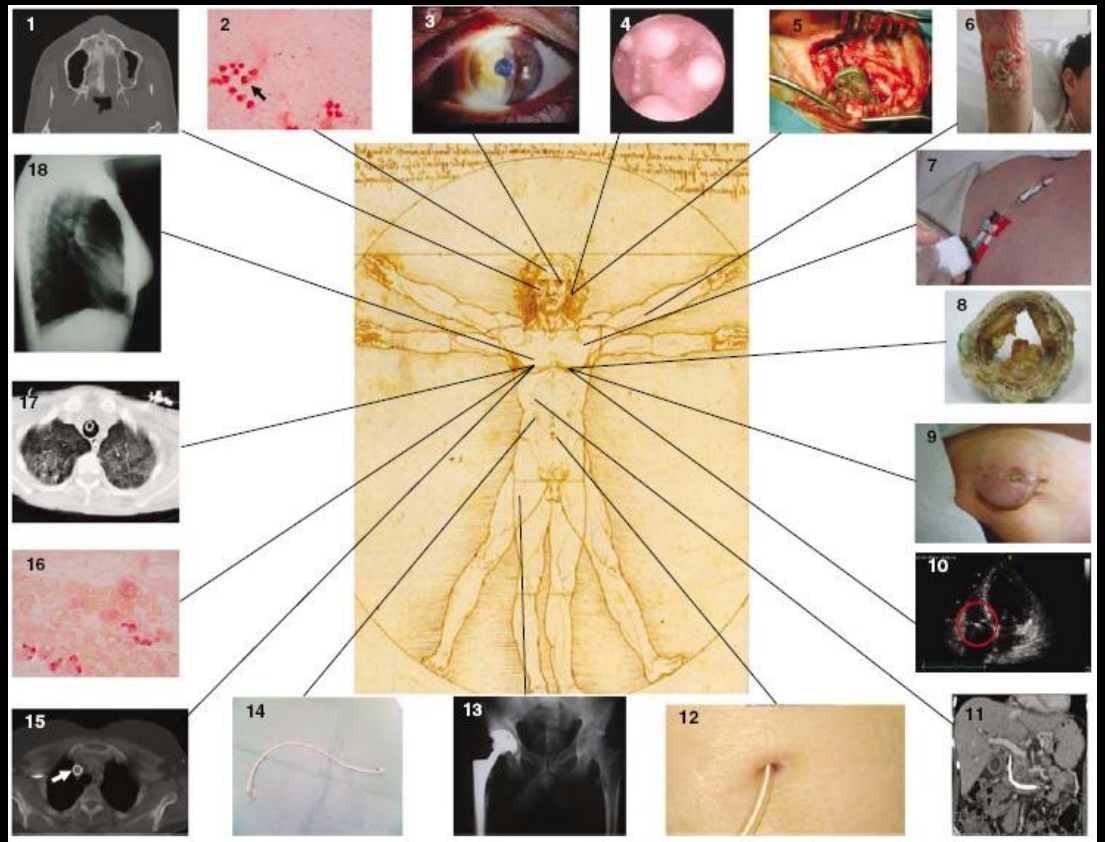
- Human Microbiome
- Oral Microbiome
- Oral Biofilms

Microbiome Existence and Functionality

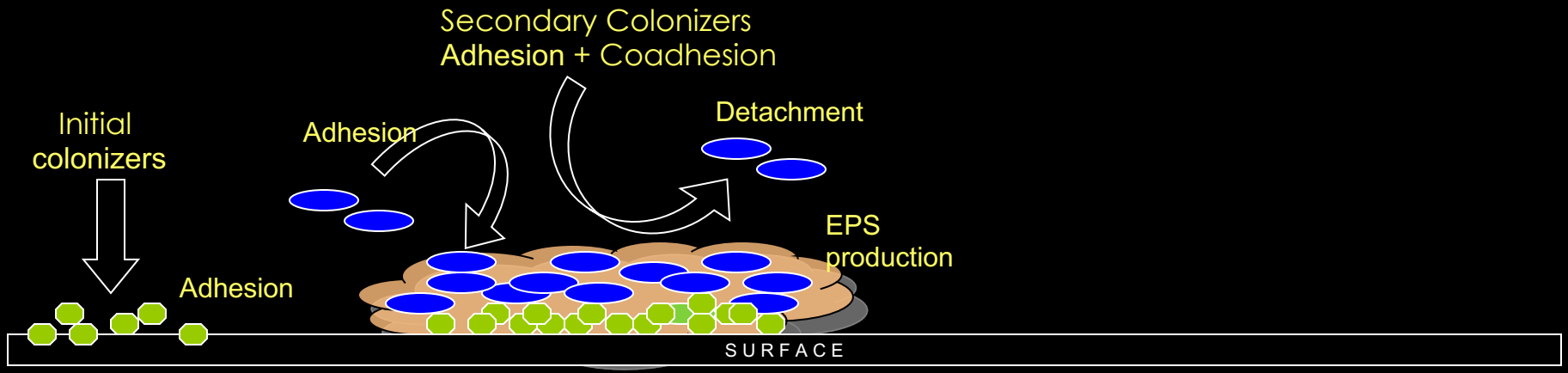
Predominant constituent of the oral microbiome is oral biofilms as opposed to the suspended/planktonic phase organisms



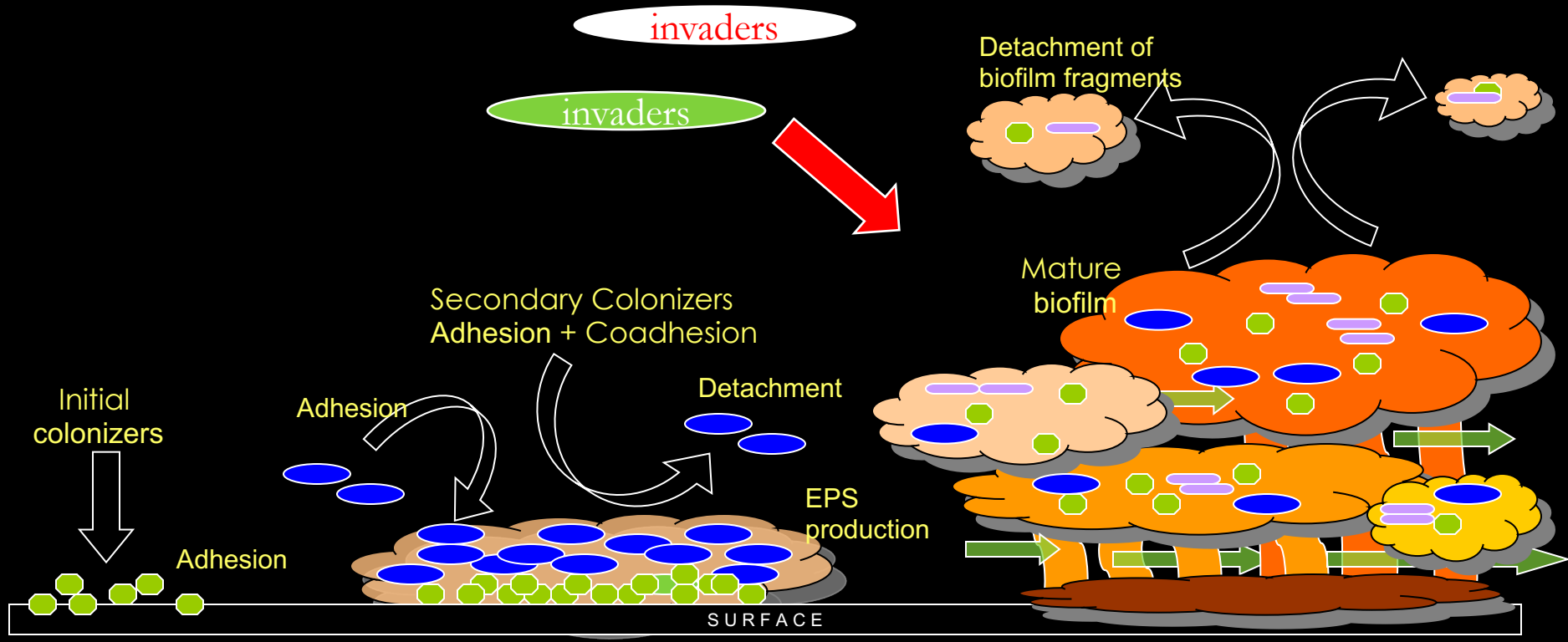
65% of all infections are related to biofilms



Biofilm Formation on Surfaces



Biofilm Formation on Surfaces



The surface of the tongue hosts a complex microbial biofilm made up of distinct clusters of bacterial species (coloured dots).Credit: S. A. Wilbert *et al. Cell Rep*. **30**, 4003–4015 (2020)

A Major Theoretical Construct

Beneficial Biofilms

vs

Pathogenic Biofilms

A Major Theoretical Construct

Beneficial Biofilms

Symbiosis

vs

Pathogenic Biofilms

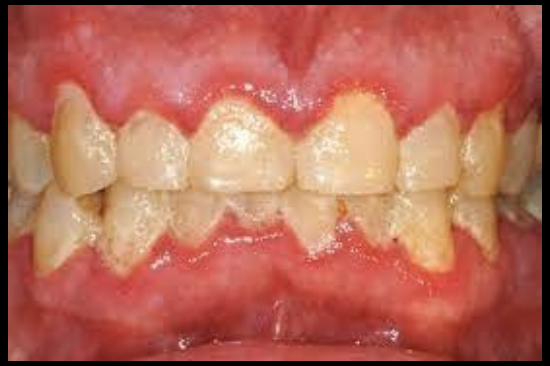
A Major Theoretical Construct

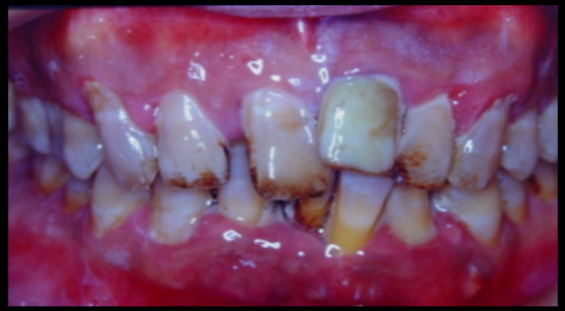
Beneficial Biofilms
Symbiosis

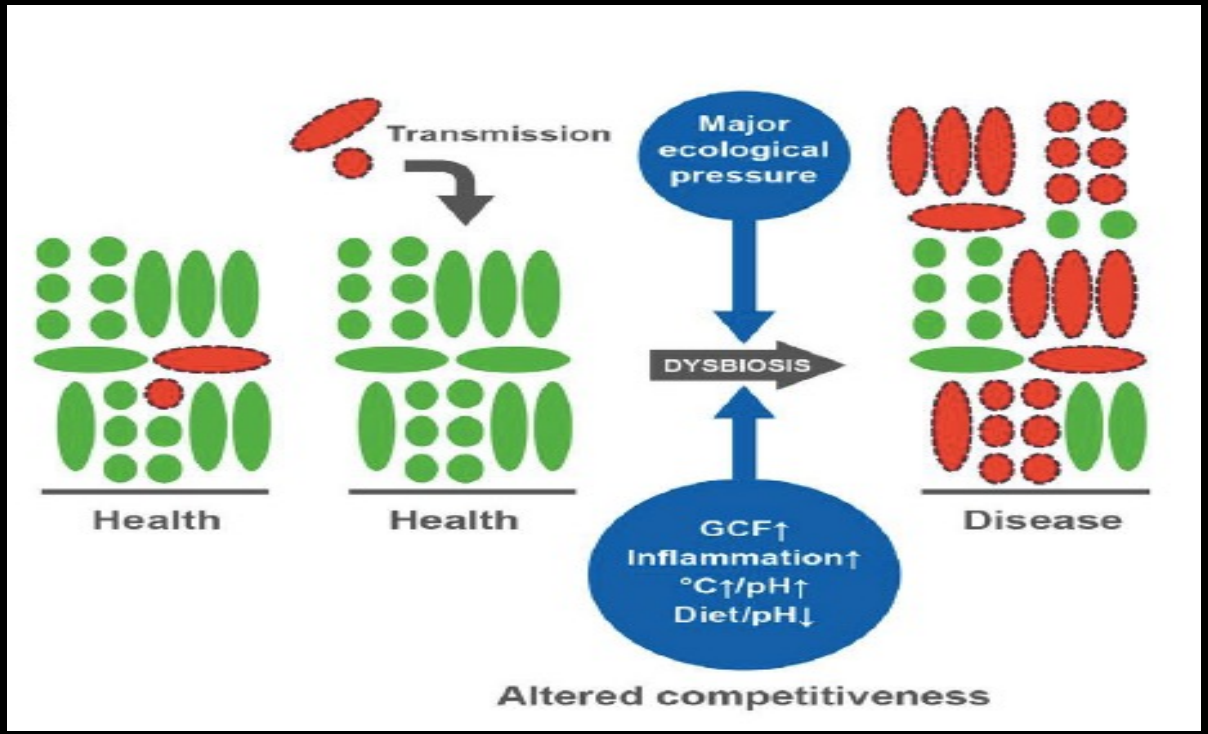
vs

Pathogenic Biofilms
Dysbiosis

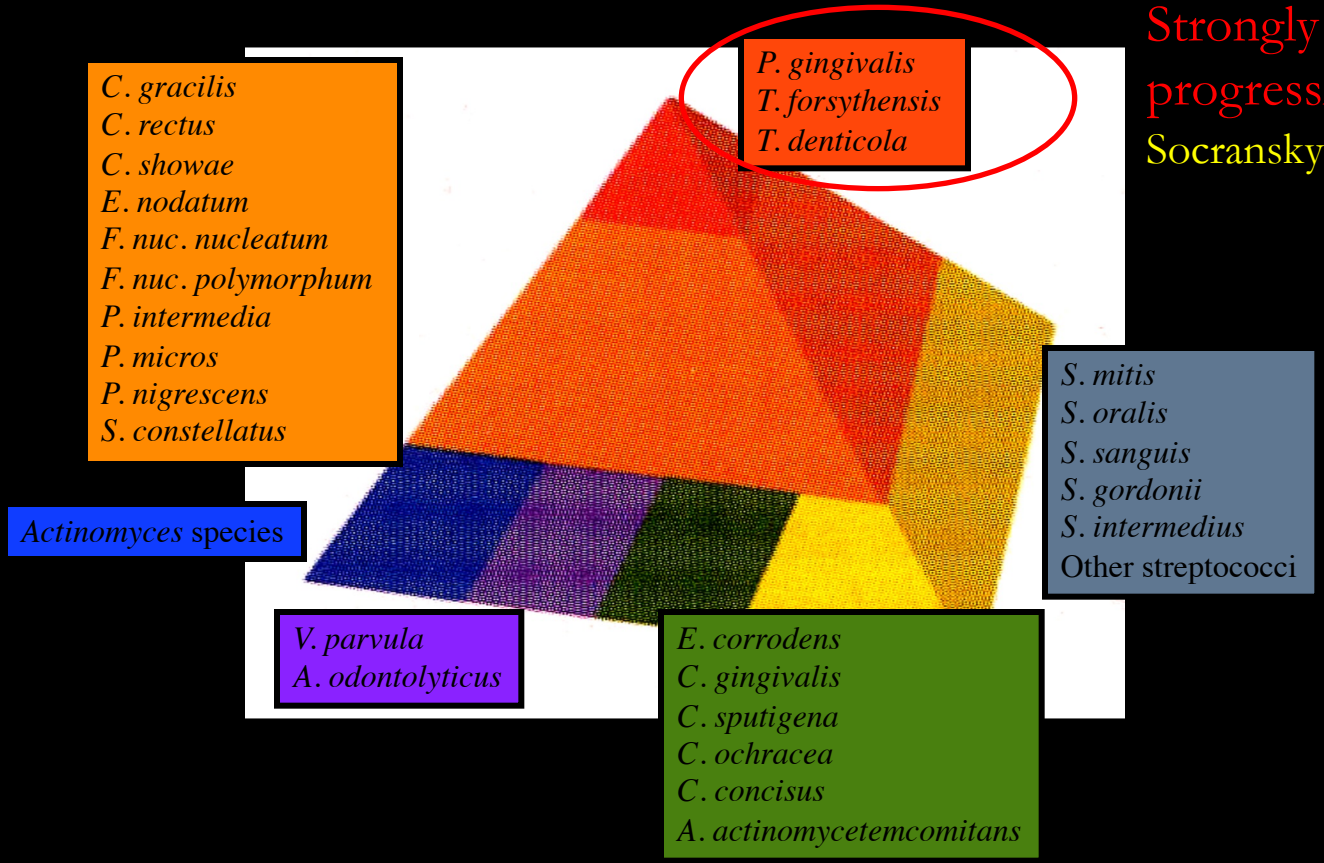
Health to Disease





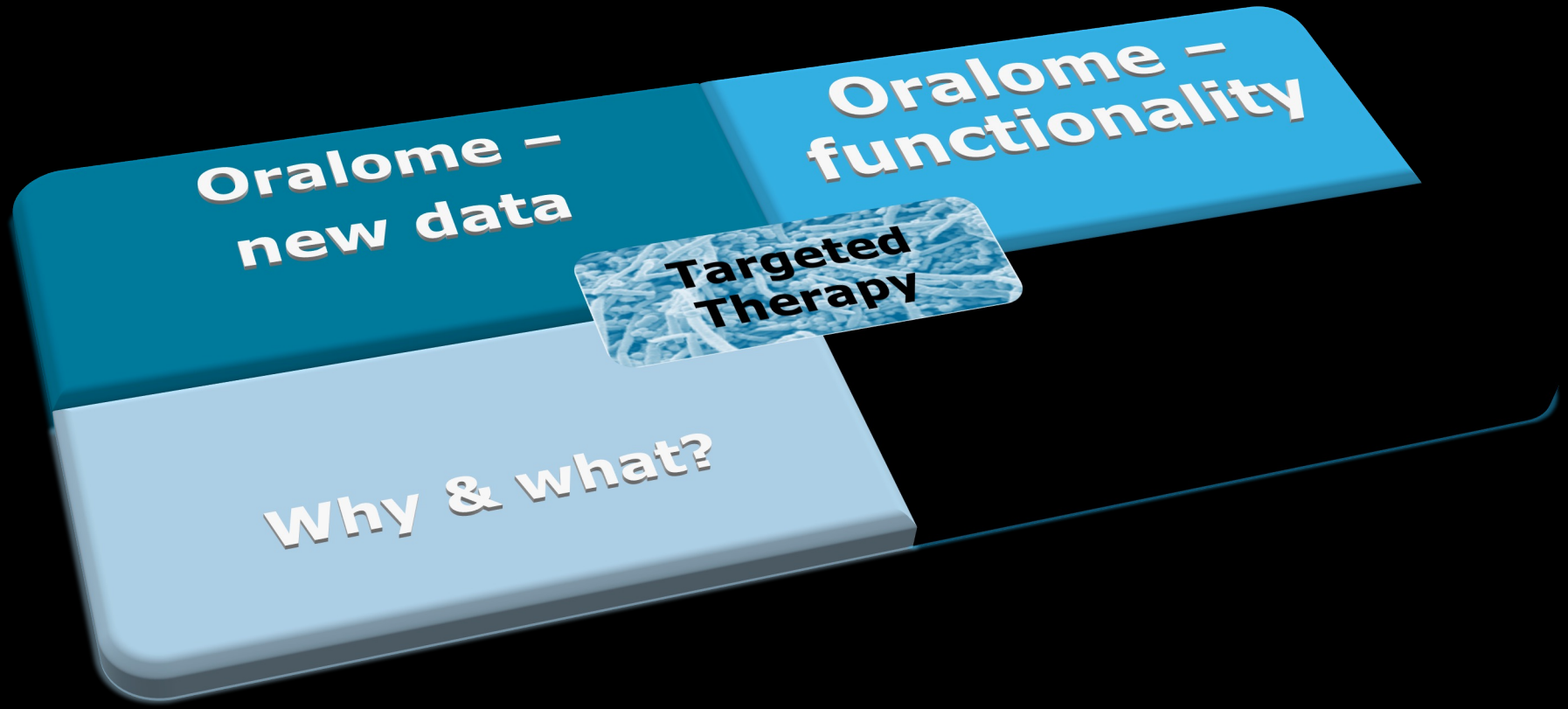

Transmission
Major ecological pressure
DYSBIOSIS
Health
Health
Disease
GCF↑
Inflammation↑
°C↑/pH↑
Diet/pH↓
Altered competitiveness

Transformation from 'beneficial biofilms' to 'pathogenic biofilm'



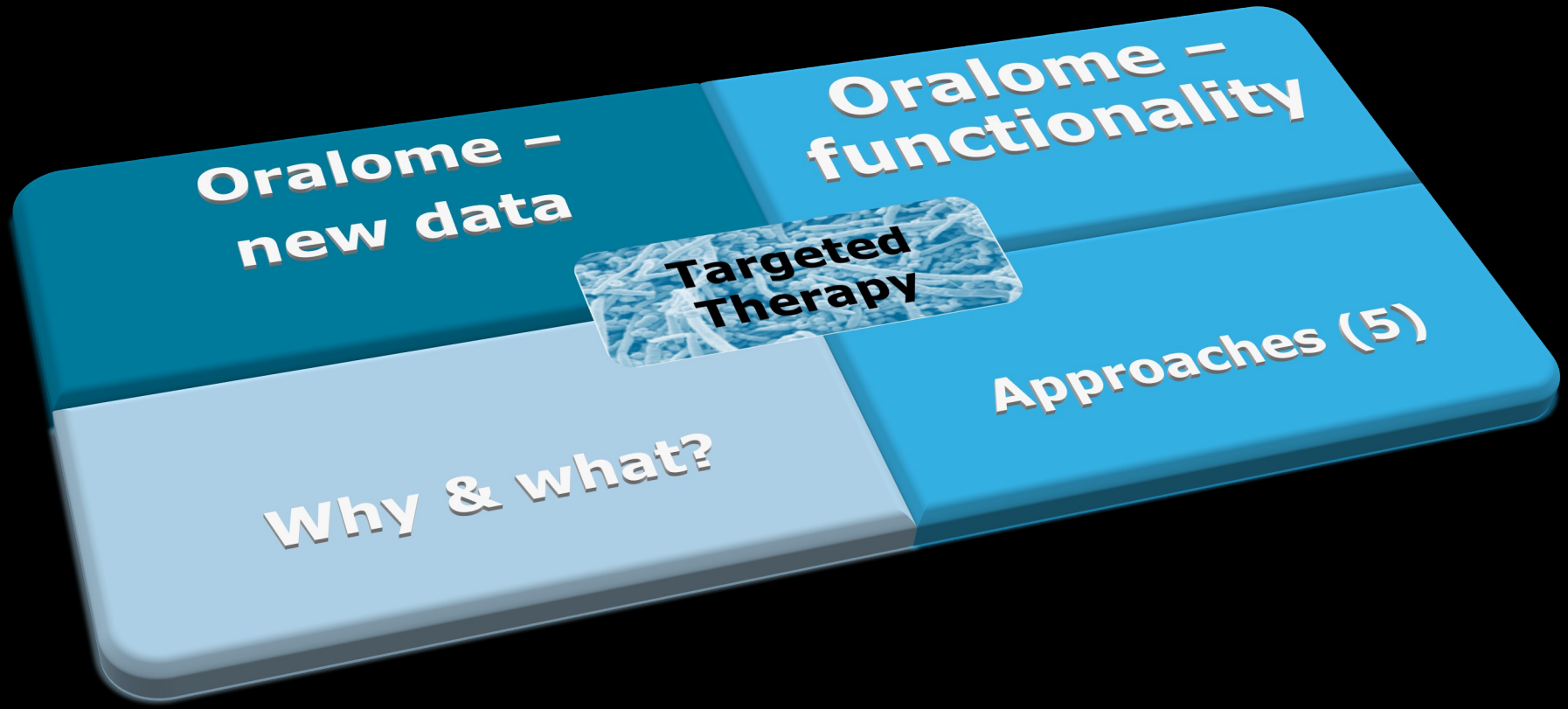
Strongly associated with progressing periodontitis.

Socransky & Haffajee. *2002*


Oralome – new data
Oralome – functionality
Targeted Therapy
Why & what?

Why targeted therapy?

- Antibiotics `carpet bomb` the commensals as well as pathogens
- Targeting a specific pathogen will tip the balance in favor of eubiosis, from dysbiosis


Oralome – new data
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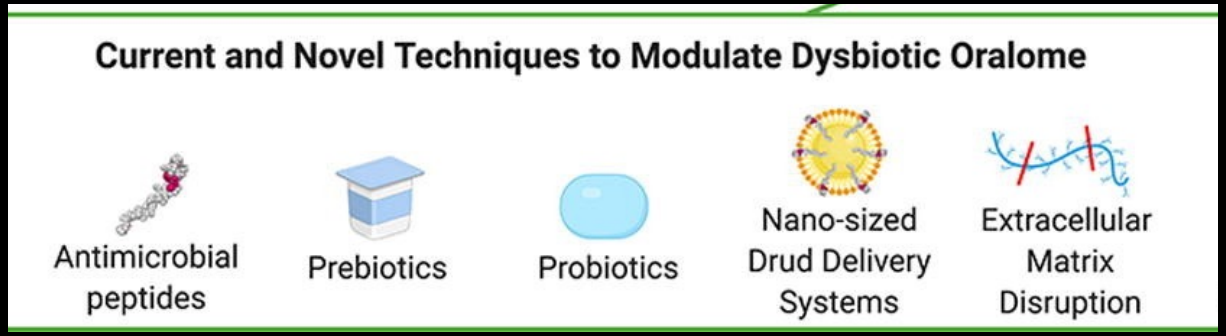
Why & what?
Approaches (5)

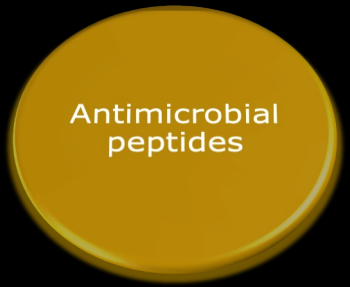
Oral Microbiota Targeted Thearpeutics

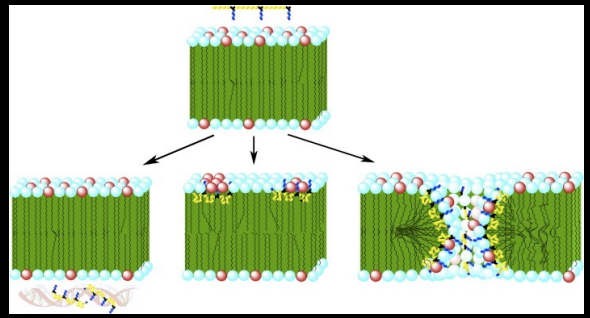



Antimicrobial peptides
Matrix disruptors
Prebiotics
Targeted Therapy
Nano drug delivery
Probiotics

I Antimicrobial Peptides (Amps)

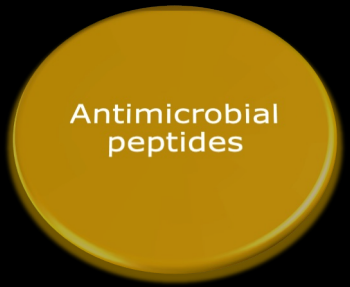
- Amps as opposed to antibiotics targets bacterial groups
- Membrane active molecules

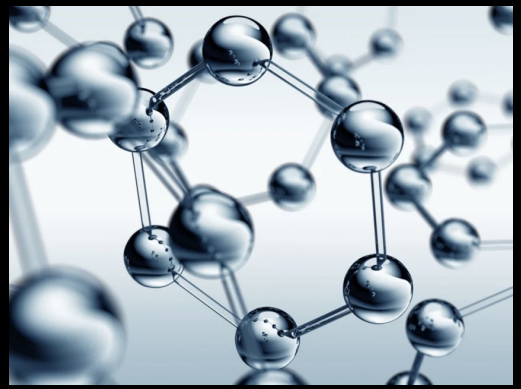




I Antimicrobial Peptides (Amps)

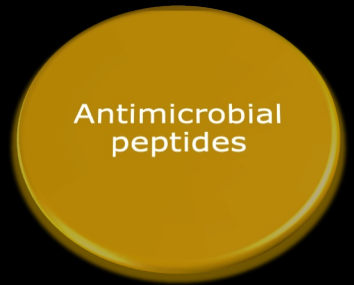
- Nisin
  - Bacteriocins like Amp produced by *Lactococcus lactis*
  - Active against both gram positive & negatives





I Antimicrobial Peptides (Amps)

- Nisin - activity
  - Disrupts oral biofilms – Inhibits planktonic growth
  - No cytotoxicity
  - Retards development of multi-species biofilms
  - Disrupts biofilm biomass and thickness in a dose-dependent manner.
  - Approved by FDA

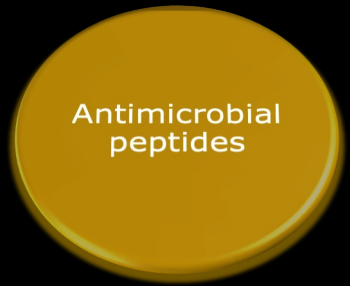


I Antimicrobial Peptides (Amps)

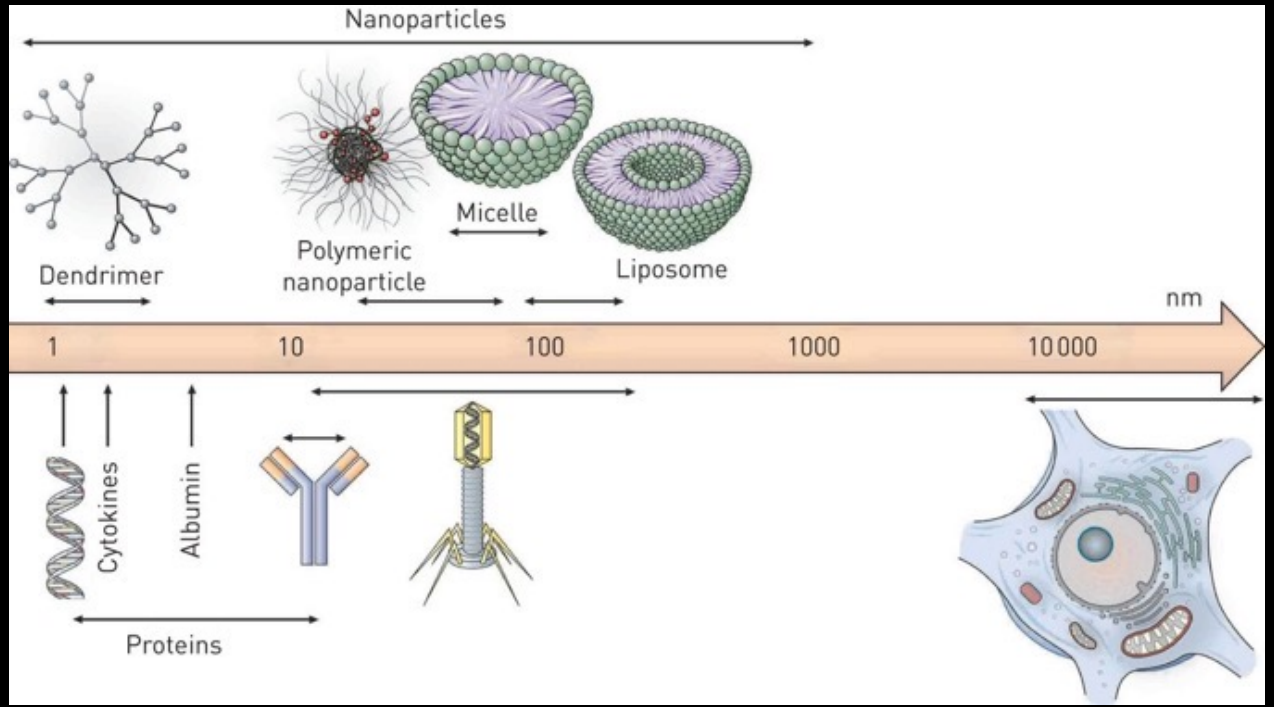
- K4-S4 Among AMPs found in amphibians, showed bactericidal effects against *S. mutans, AA* and *F. nucleatum*
- Peptoids
- Arylamide oligomers
- Beta- peptides

Challenges

- Resistance development
- Act as host sensitizers/allergens,

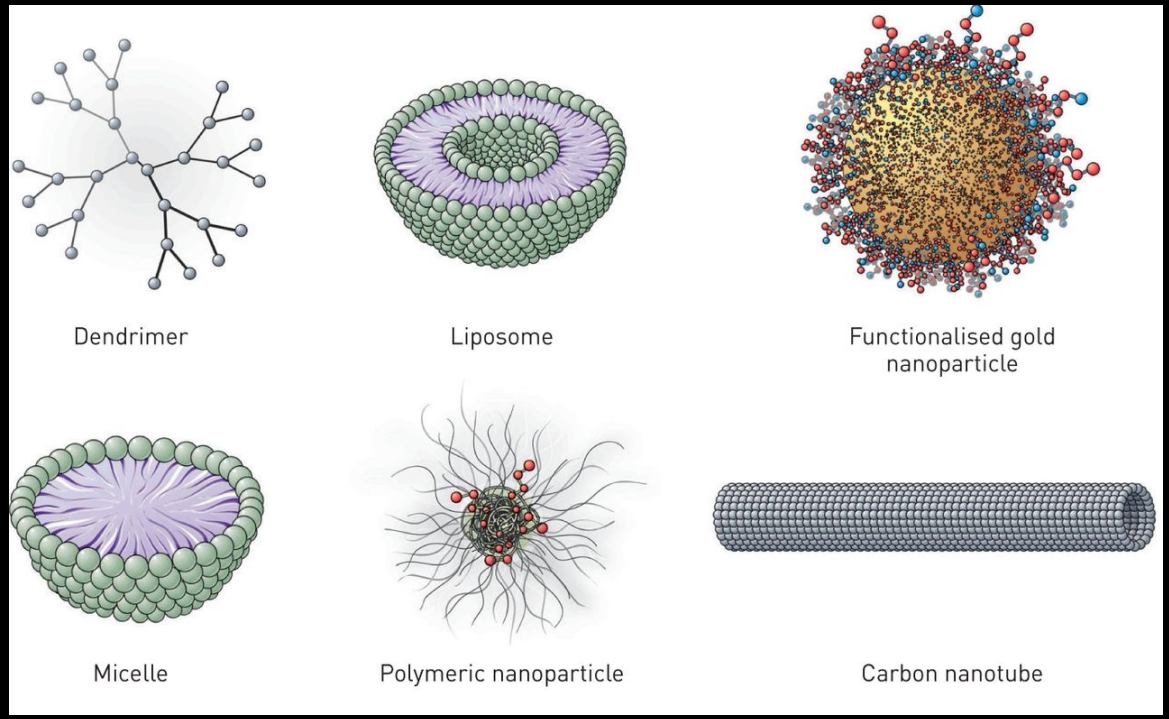


II Nanosized-drug Delivery Systems



Colloidal particles sizes between 1 and 1000 nm ; Smaller the size better the activity

II Nanosized-drug Delivery Systems



II Nanosized-drug Delivery Systems

- Nanoparticles of Ag, Cu, TiO_2, ZnO incorporated into polymer matrices
- A polymeric nano-DDS capable of releasing quorum sensing chemical - farnesol releasing the drug only at acidic pHs
- This disrupt only the aciduric dysbiotic biofilm. (Horev et al.)
- Potential in caries reduction

II Nanosized-drug Delivery Systems

- Nano-DDS disrupt *S. mutans* biofilms 4-fold more effectively than the free drug *in vitro*
- Reduces both the number and severity of carious lesions *in vivo,* compared to free farnesol
- Nano-DDS protect AMPs from degradative enzymes and help evade the host response

III Prebiotics

- Definition: Natural or synthetic food ingredients or supplements that modulate the microbiome to benefit the host
- Common in managing gut microbiome but recently applied to oral microbiome
- Common Prebiotics
  - Arginine
  - Nitrate
  - Mannosamines

III Prebiotics

- **ARGININE**
  - Prevent caries by buffering the acids produced by a dysbiosis (Agnello et al.)
- NITRATE
  - Useful against both caries and periodontitis by increasing ammonium production and increasing pH (Rosier et al.)
- MANNOSAMINE
  - Selectively triggered the growth of commensal oral bacteria and shifts biofilm communities towards a eubiosis in vitro (Slomka et al.)

Many more additional studies needed!

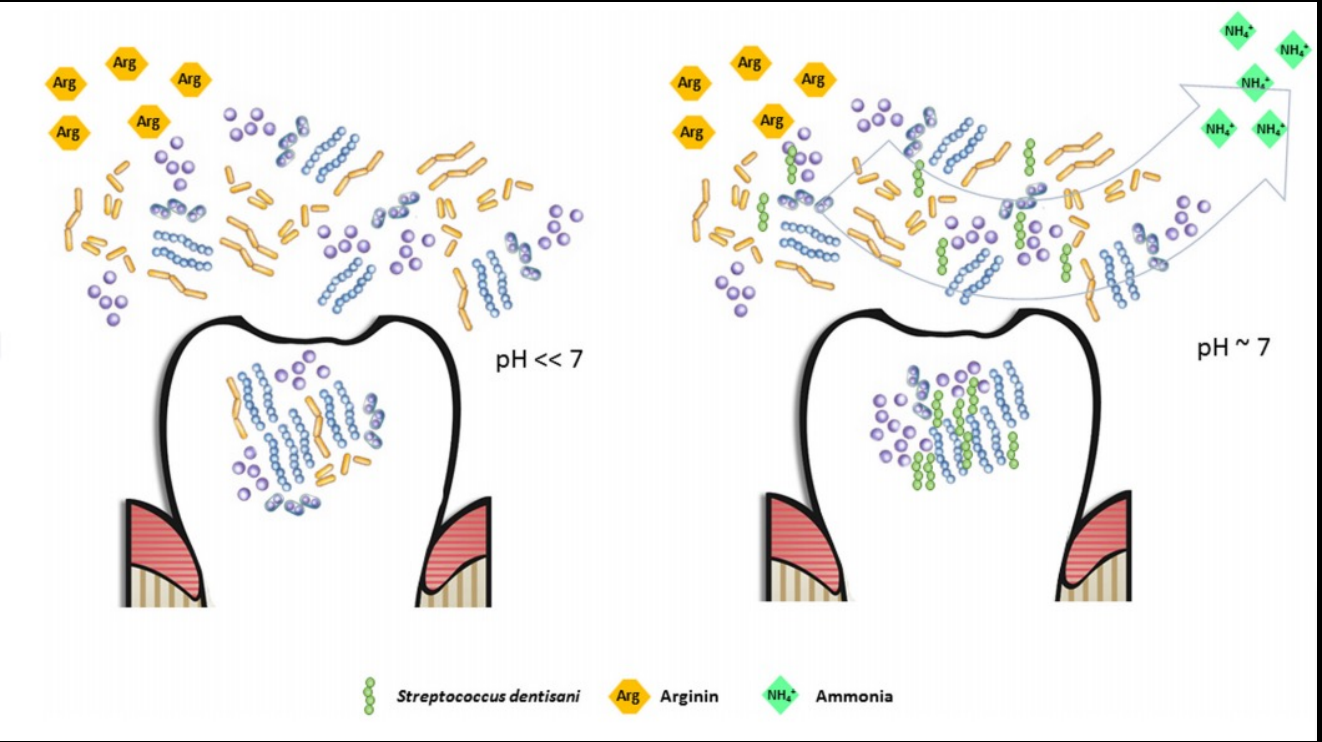
IV Probiotics

- Definition: Live microbes which, when administered in adequate amounts, confer a health benefit for the host
- Lactobacilli and streptococci from healthy oral cavities have antibacterial activity against :
  - *P. gingivalis,*
  - *P. intermedia,*
  - *A. actinomycetemcomitans*
  - *F. nucleatum*

IV Probiotics

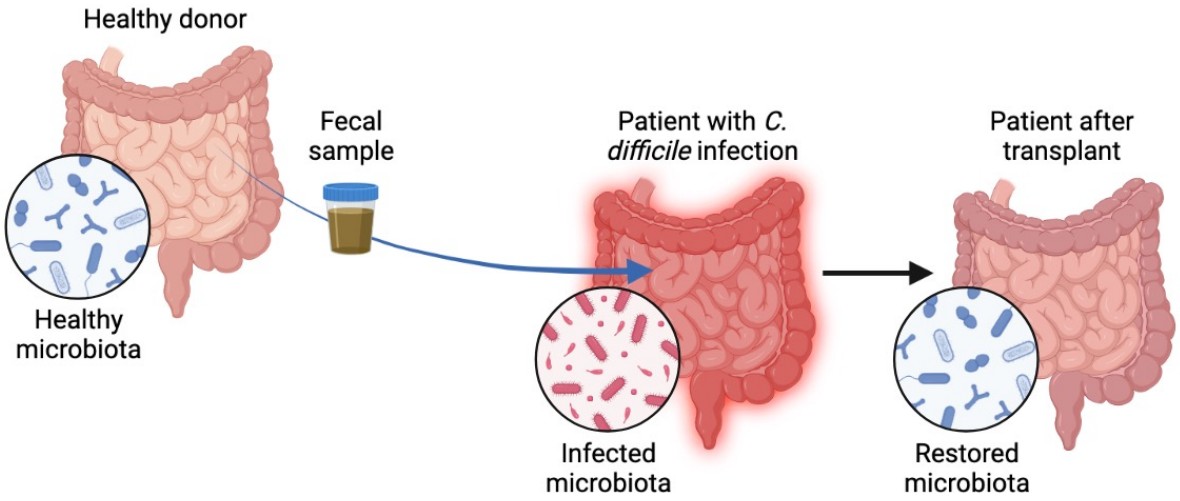
- Pre-clinical and clinical studies indicate that probiotics reduce
  - Gingivitis
  - Plaque
  - Alveolar bone loss
  - Modulate pro-inflammatory effects
- Specifically, chewing gum with *Lactobacillus reuteri* led to reduction in gingivitis and plaque in patients with moderate to severe gingivitis
- *Bacillus subtilis* reduced bone loss and attachment loss in rats with ligature-induced periodontitis

Case of *Strep. dentisanis*



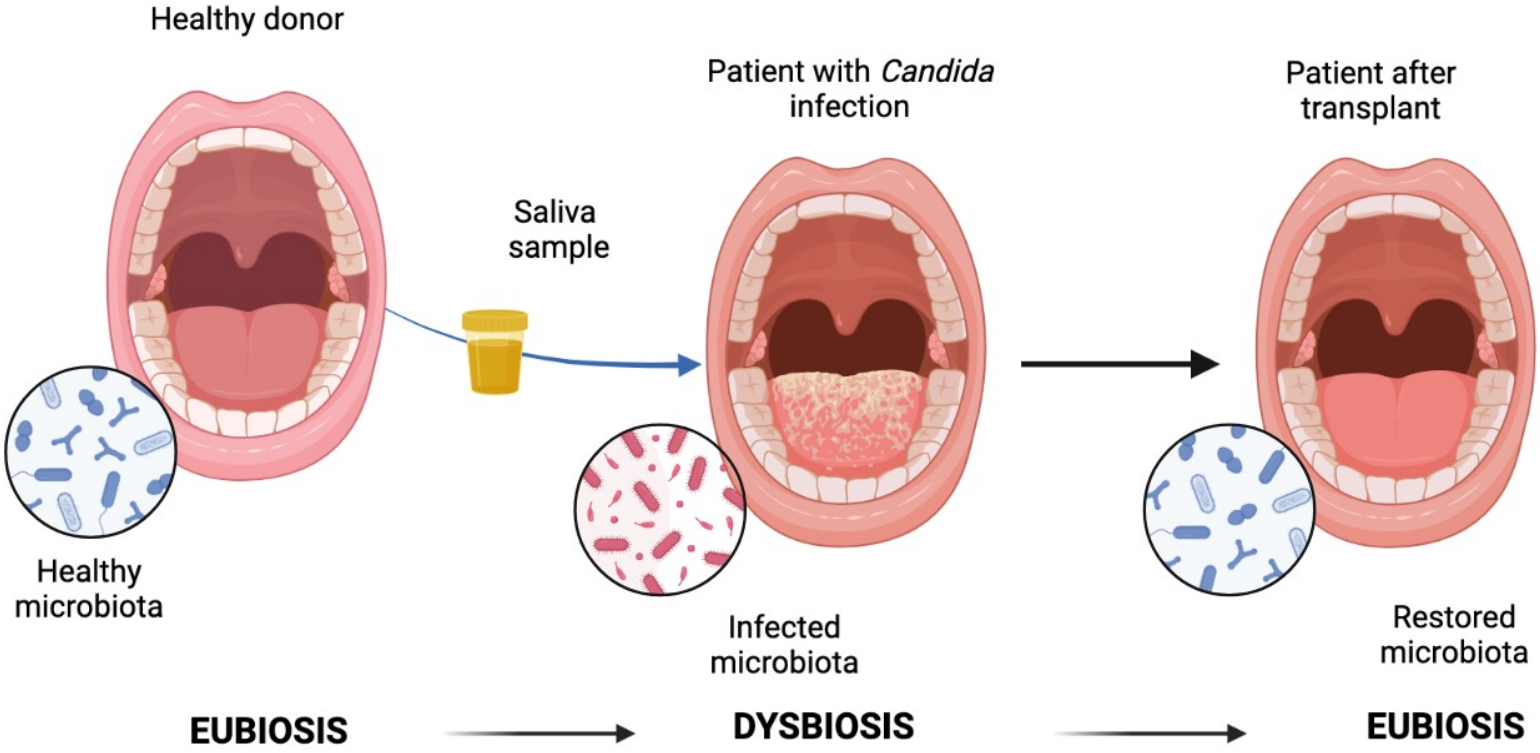
López-López, Arantx.; *et al.* Health-Associated Niche Inhabitants as Oral Probiotics: The Case of *Streptococcus dentisani*. *Frontiers in Microbiology*. 2017, 8(119).

Gut Microbiota Restoration in Diseased Patients



Prepared with BioRender.com

Oral Microbiota Restoration in Diseased Patients



IV Probiotics - drawbacks

- A large number of probiotic cells needed for a prolonged periods in target site
- Transport in viable form is difficult due to survival issues
- Thus, encapsulation of probiotics in micro- and nano-sized drug delivery systems (nano-DDS) may be an alternative (Koo et al.)

V Extracellular polymeric matrix (EPM) disruption

- EPM is the matrix of the oral biofilm

- Two main strategies to disrupt EPM
  - Disrupting EPS synthesis
  - Disrupting secretion and targeting

  its composition and structure

V Extracellular polymeric matrix (EPM) disruption

- Disrupting EPS synthesis and secretion has been achieved by
  - cyclic-di-GMP or cyclic -di-AMP (Karaolis et al., Yan et al.)
- Disrupting EPS composition/structure achieved by the use of exopolysaccharide-degrading enzymes
  - Dextranase
  - Mutanase
  - Dispersin B

Top five

Take Home Messages

Top 5 Messages

- Oralome is far more complex than we thought!
- **Multi-drug resistance antibiotics** increase the urgency for development of targeted therapy
- Multitude of approached for targeted disruption of pathogens to convert **dysbiosis to eubiosis**
- Targeted disruption of microbes can be used to **replace antibiotics** in future
- Caries and POD are likely to be amenable to targeted therapy

Good Luck & Thank You!

Please send comments/queries to:

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