BIOFILM

ITS ROLE IN PERIODONTAL DISEASE

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Reader

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The universe is not only queerer than we imagine,

It is queerer than we can imagine

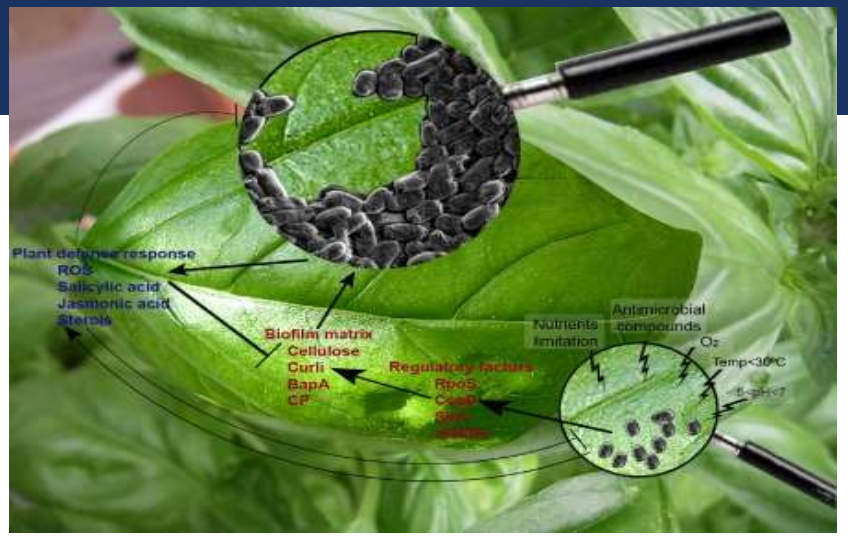
- JBS Haldane

Biofilms are matrix enclosed bacterial populations adherent to each other or/to surfaces or interface.

- Costerton, 1994

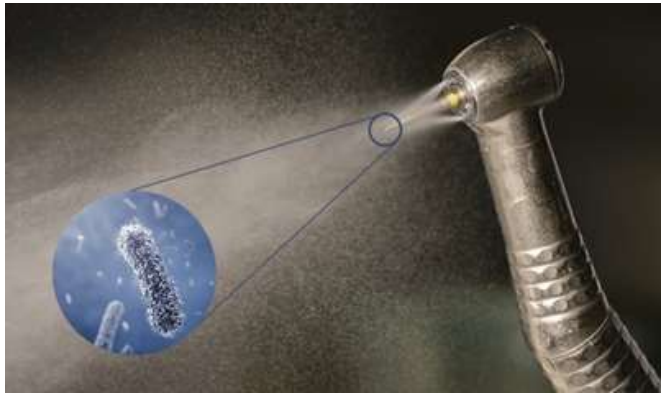
Biofilms are ubiquitous

BIOFILM IN NATURE

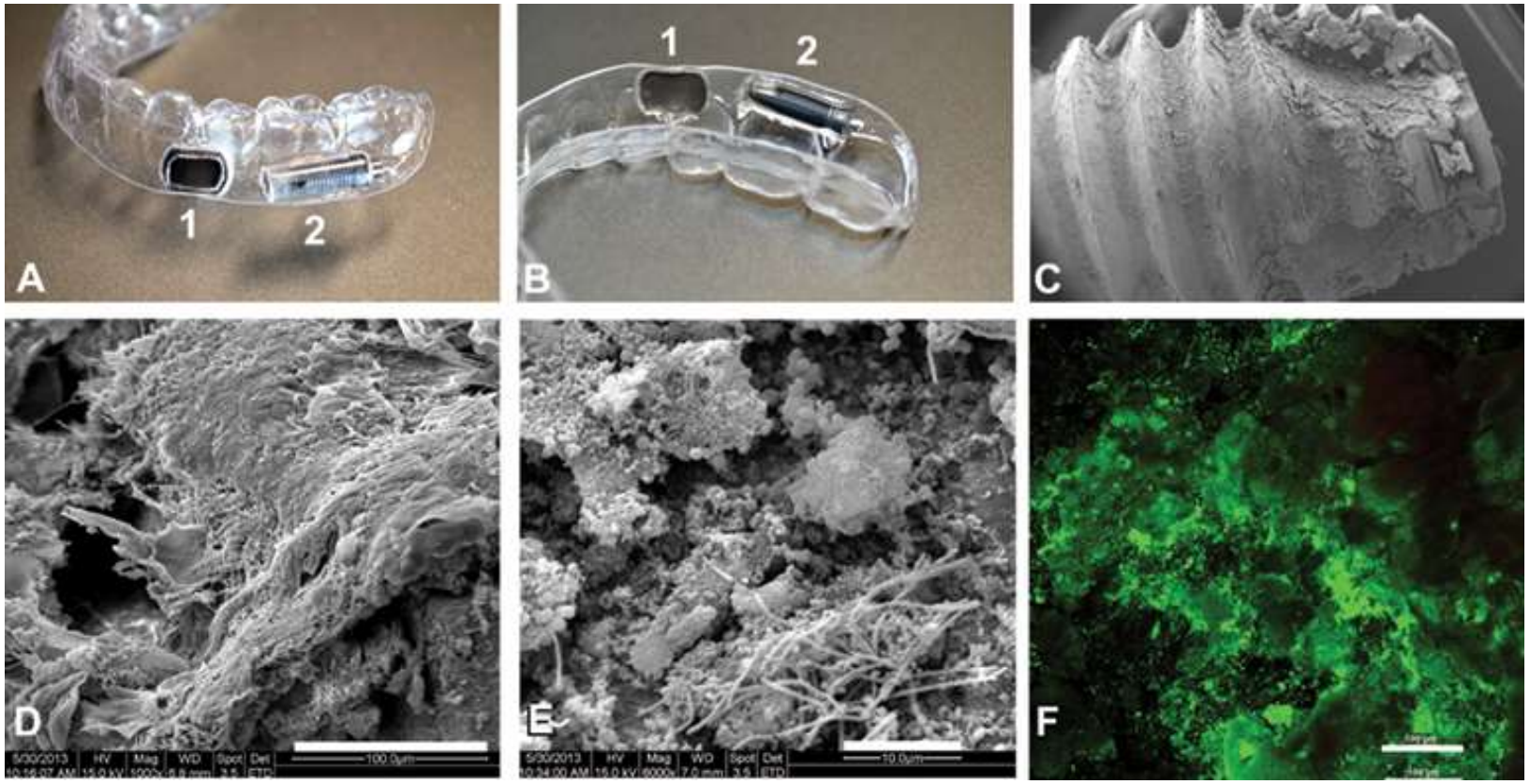


BIOFILM IN HOUSEHOLD

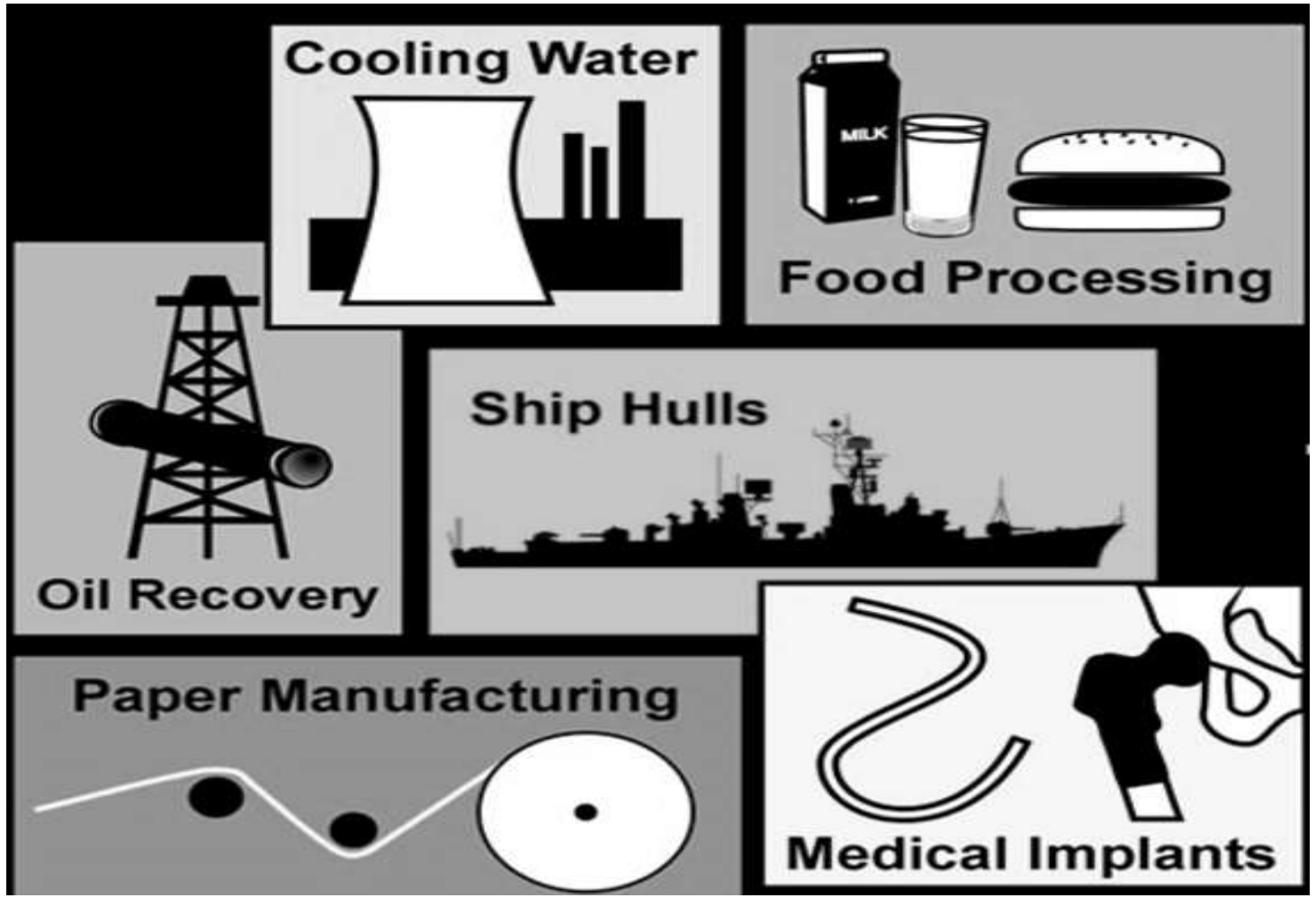
BIOFILM IN DENTAL UNITS



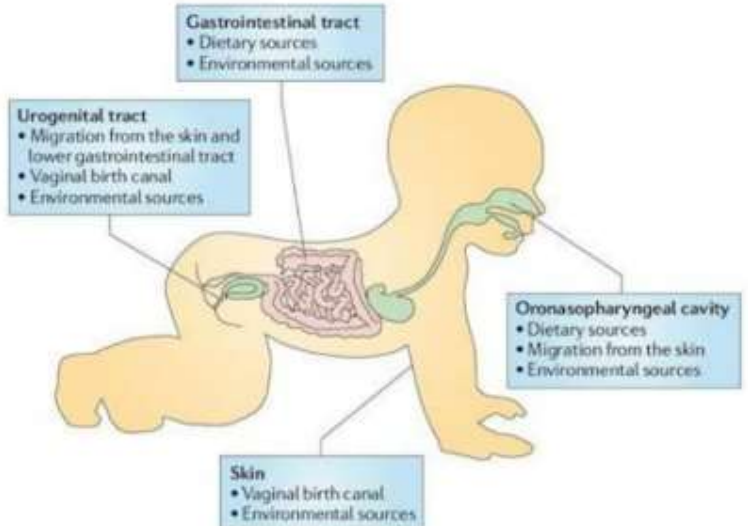
BIOFILM ON DENTAL IMPLANT AND PROSTHESIS

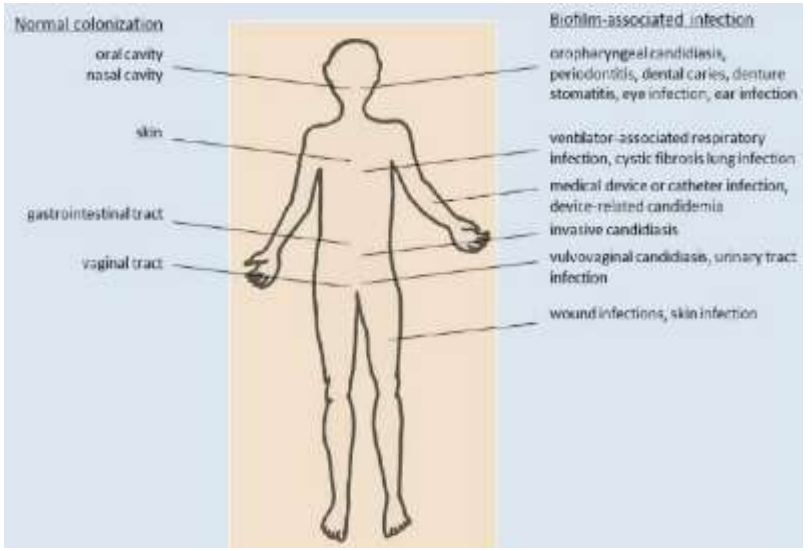


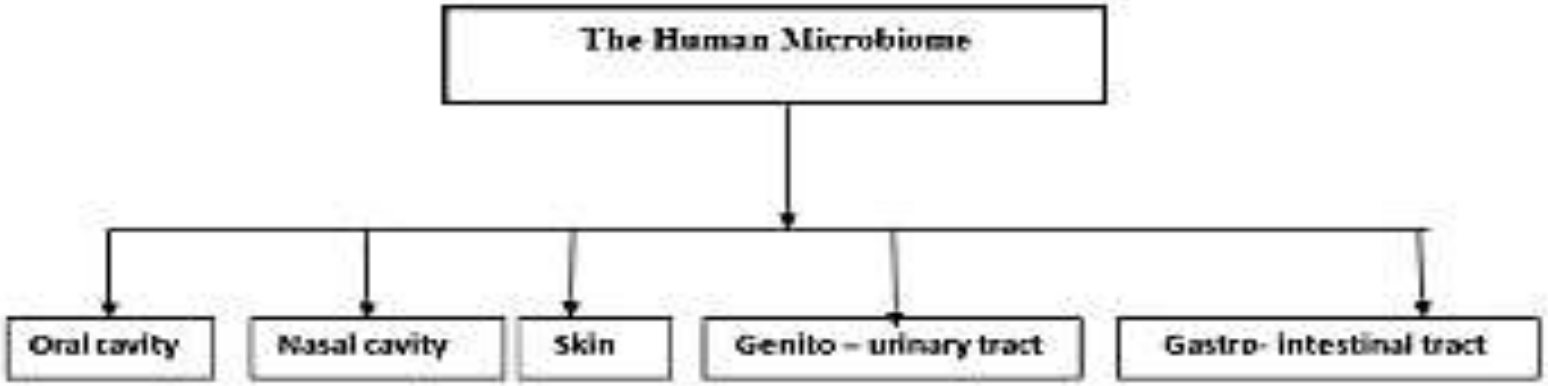
BIOFILMS IN INDUSTIRES



HUMAN MICROBIOME

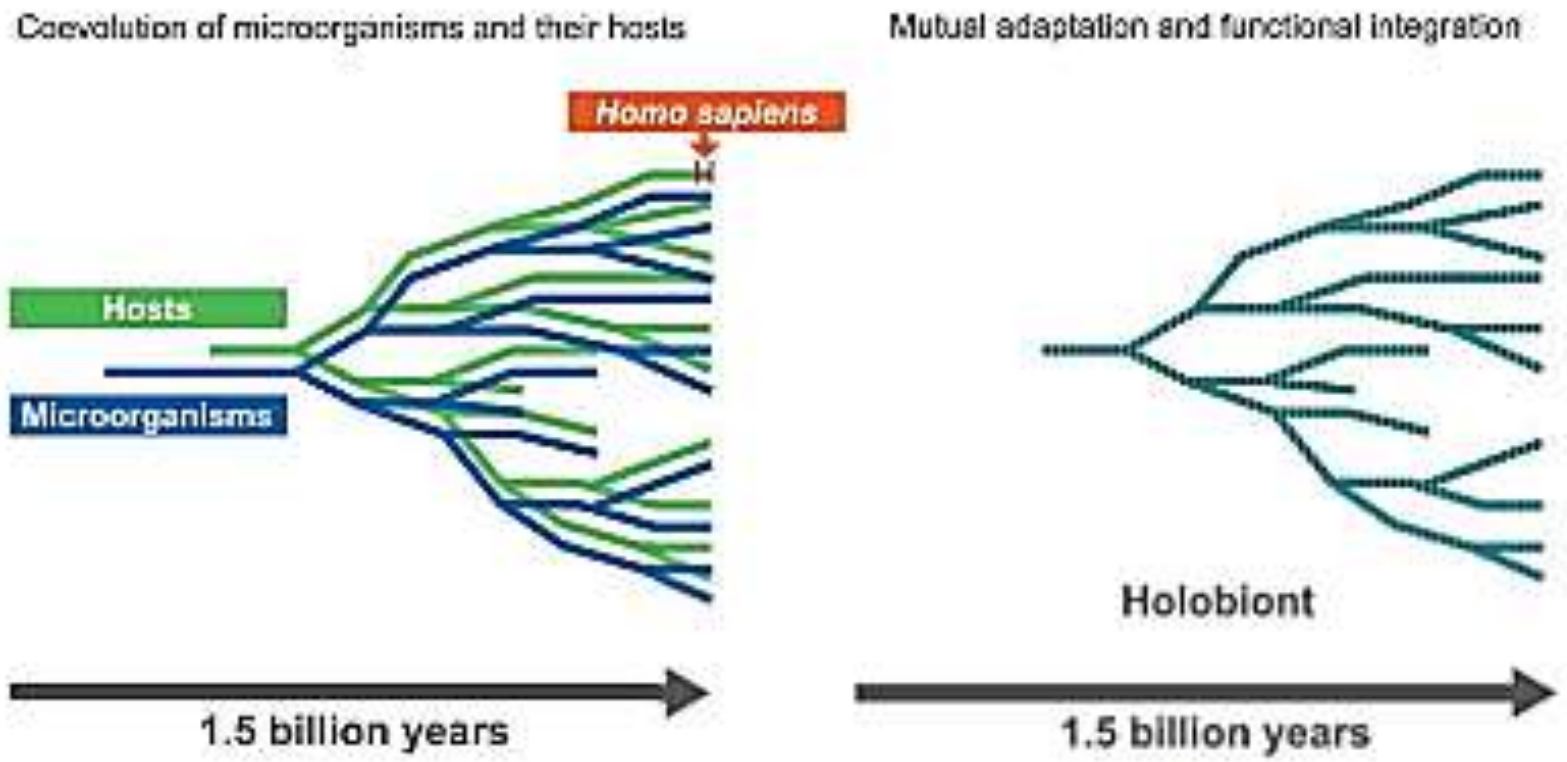




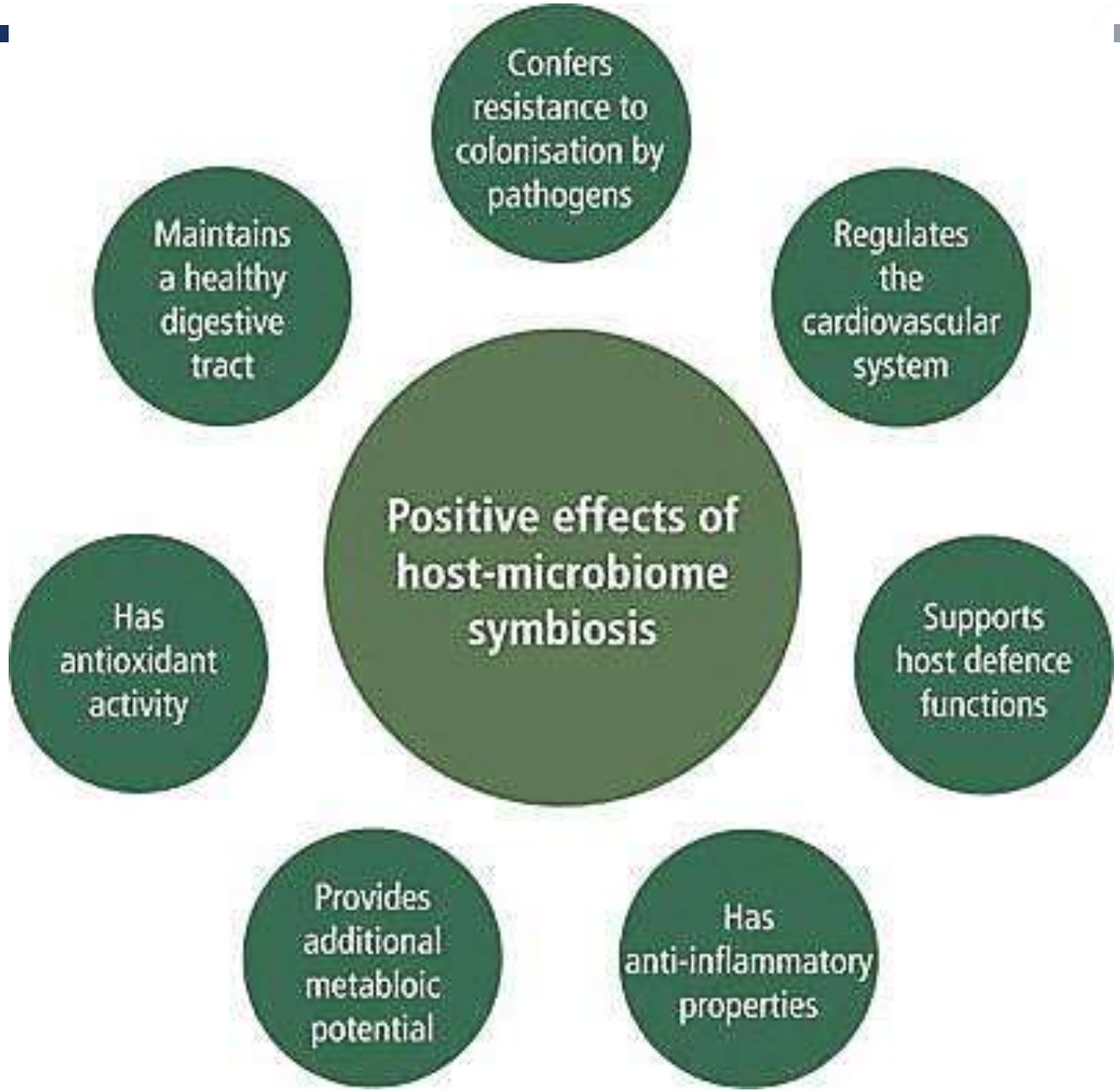


Number of Cells in the human body 10 trillion; only 10% are human and the rest are microbial

HOLOBIONT



History of the relationship between microorganisms and humans: The coevolution between microorganisms (blue lines) and their respective hosts (green lines) over a period of 1.5 billion years has resulted in mutual adaptation and functional integration as reflected in our own relationship to most of the microorganisms that colonise our body surfaces (*Homo sapiens*, red line)

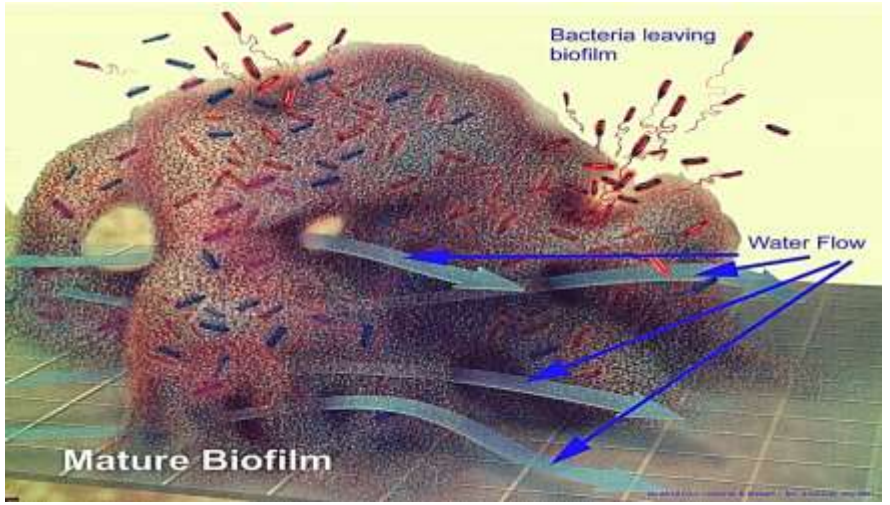


-M Kilan et al, 2016

PLANKTONIC BACTERIAL CELL IS A HOMELESS VAGABOND

BIOFILM IS A SURVIVAL STRATEGY

Planktonic



Biofilm

Biofilms are home for microorganisms

Lindhe 5th edition

BENEFITS OF BIOFILM TO MICROORGANISMS

BENEFIT OF BIOFILM TO MICROORGANISMS

Community life-style in biofilms provides enormous potential benefits to the participating organisms.

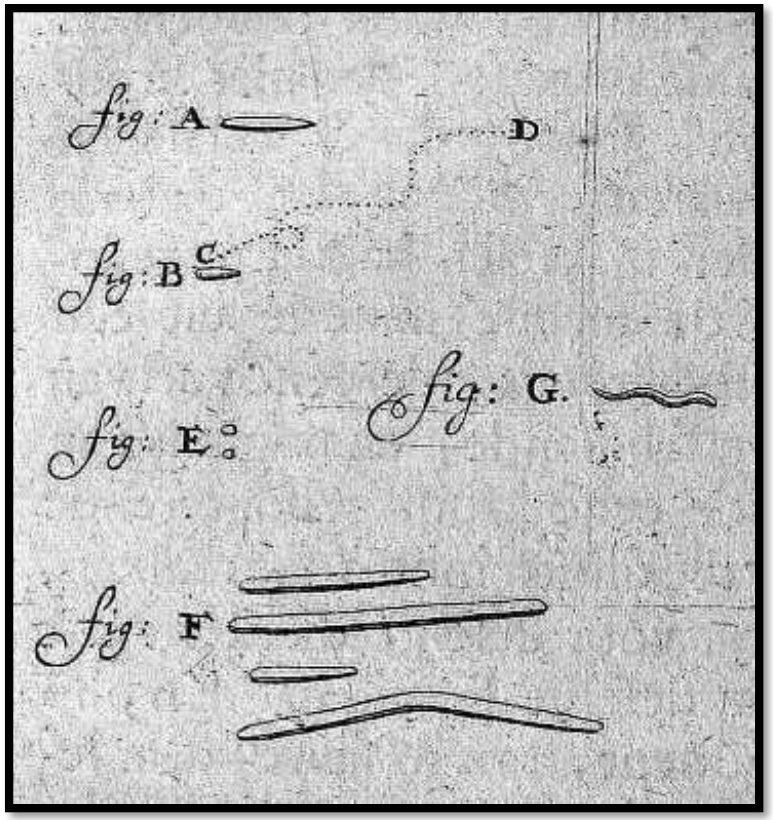
- A broader habitat range for growth.
- An increased metabolic diversity and efficiency
- An enhanced resistance to environmental stress, antimicrobial agents and the host defences
- It afford physical protection from phagocytosis for cells deep within a spatially organized consortium
- An enhanced ability to cause disease

BIOLOGICAL INSURANCE

The *structural* and *environmental* heterogeneity of the biofilm accelerates the *phenotypic* and *genotypic* diversity in bacterial population and might be a mechanism where by cells are better prepared to cope with adverse conditions. It is termed as biological insurance.

-Boles et al 2004

DENTAL PLAQUE IS A TOOTH ASSOCIATED BIOFILM

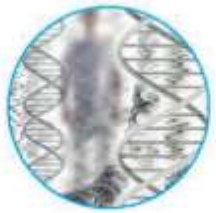


On september 17, 1683, leeuwenhoek wrote to the royal society about his observations on the plaque between his own teeth, "*a little white matter, which is as thick as if 'twere batter*. In the mouth of one of the old men, leeuwenhoek found "*an unbelievably great company of living animalcules, a-swimming more nimbly than any I had ever seen up to this time. The biggest sort. . . Bent their body into curves in going forwards. . . Moreover, the other animalcules were in such enormous numbers, that all the water. . . Seemed to be alive.*

MILESTONES IN ORAL MICROBIOLOGY

1800s
Connection made between disease and microorganisms

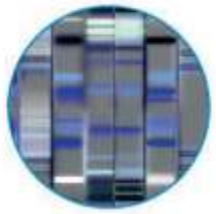
1980s–present
Unculturable microorganisms studied using DNA sequencing



2010
Human Oral Microbiome Database launched

Mid-1600s
First observations of microscopic organisms

Late 1800s–present
Culture, staining and microscopy used to study microroorganisms



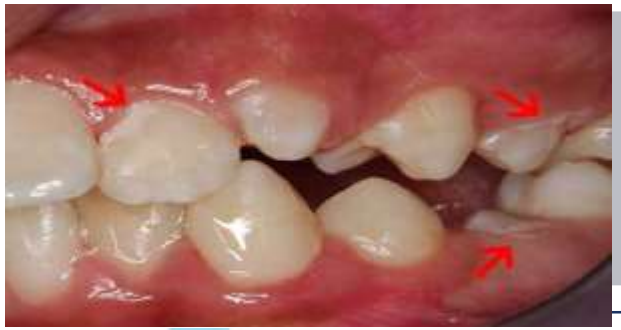
2008
Human Microbiome Project launched to characterize the human microbiome

ORAL CAVITY – AN IDEAL MICROBIAL HABITAT

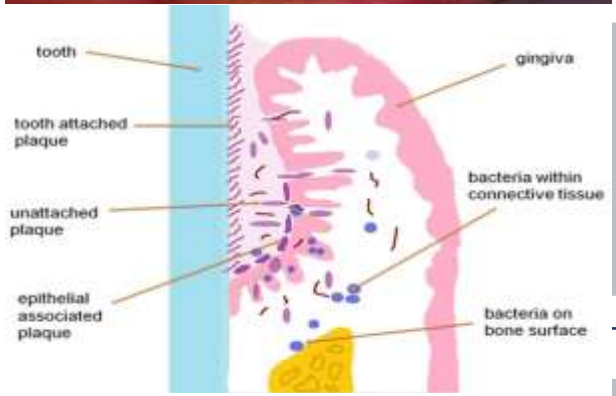
ORAL CAVITY

- Oral Cavity is warm and moist.
- Temperature: 35 to 36 degree centigrade
- pH: 6.75 to 7.25
- Saliva and GCF : Nutrient rich bulk fluids
- It provides non shedding surfaces in the form of tooth and restorations.
- Tongue, tonsils, pocket are good soft tissue habitats.

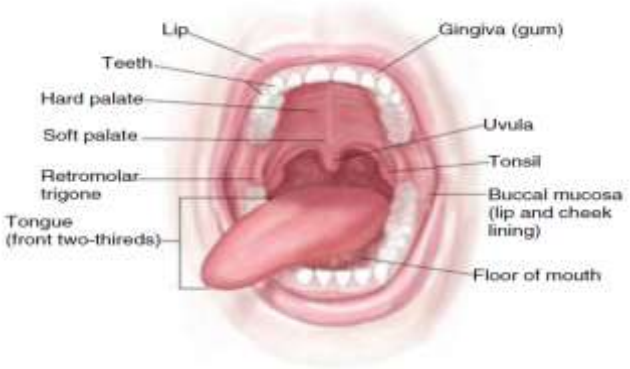
On the basis of physical and morphological criteria, the oral cavity is divided into six major ecosystems:-



Supragingival surfaces



Subgingival surfaces



Floor of the mouth

Tonsils

Tongue

Saliva

DEFINITION OF PLAQUE

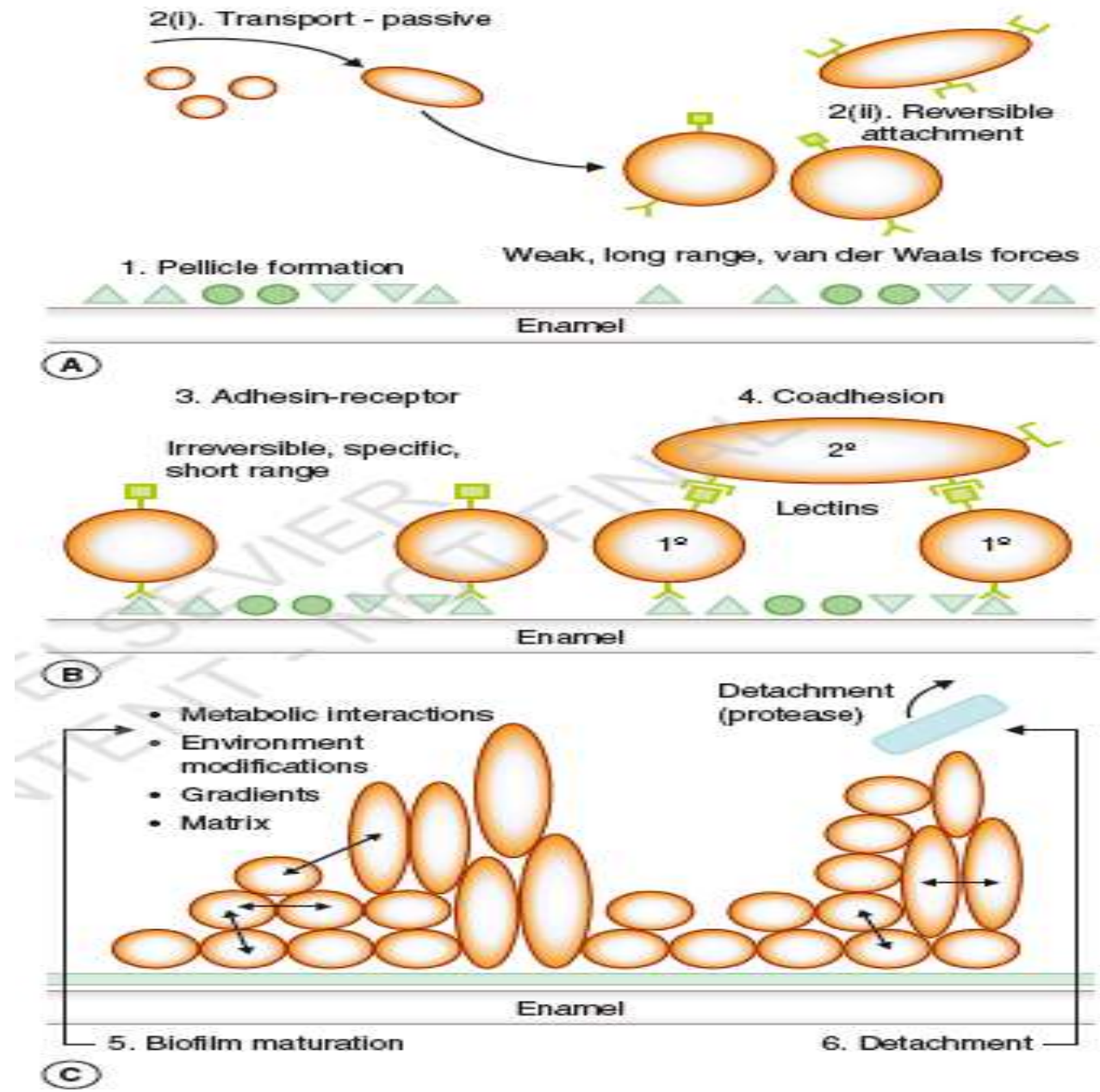
- Dental plaque is defined as a complex microbial community that develops on tooth surfaces embedded in a matrix of polymers of bacterial and salivary origin.

- Philip D Marsh, 1976

Biofilm formation

Steps in Biofilm formation

1. Pellicle Formation
2. Reversible Attachment
3. Irreversible Attachment
4. Co Aggregation
5. Biofilm Maturation
6. Detachment & Dispersion


2(i). Transport - passive
2(ii). Reversible attachment
Weak, long range, van der Waals forces
1. Pellicle formation
Enamel
A
3. Adhesin-receptor
Irreversible, specific, short range
4. Coadhesion
2º
Lectins
1º
1º
Enamel
B
Detachment (protease)
• Metabolic interactions
• Environment modifications
• Gradients
• Matrix
Enamel
5. Biofilm maturation
6. Detachment
C

STEP 1:ACQUIRED PELLICLE FORMATION

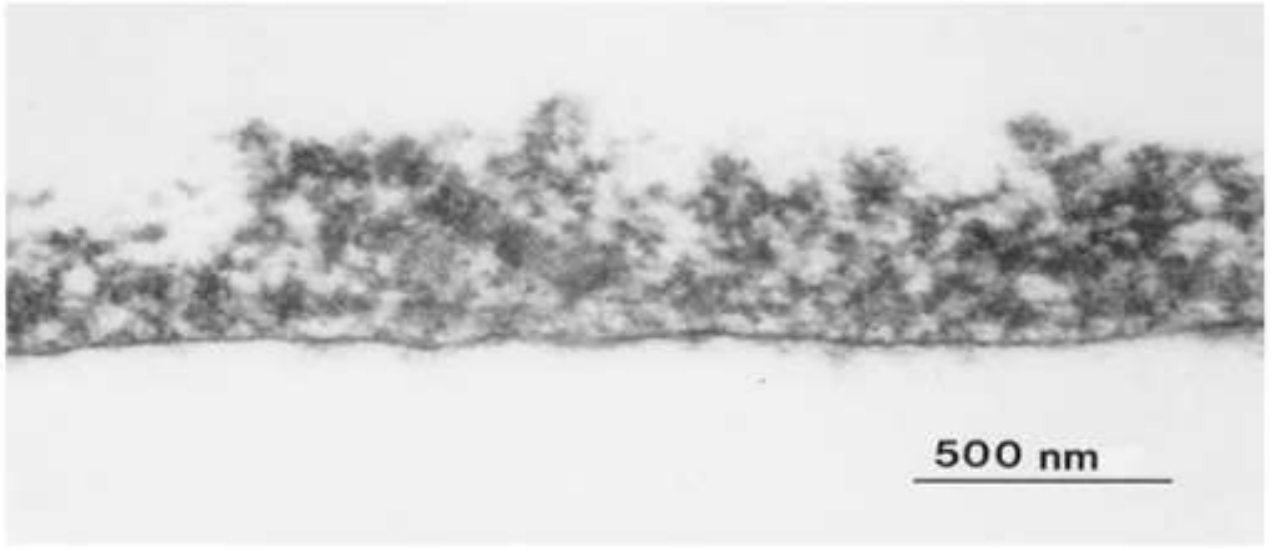
- Acquired pellicle is protienous film that forms on the surface of tooth by selective binding of salivary glycoproteins which forms in seconds after teeth are clean.

- The major constituents of pellicle are salivary glycoproteins, phosphoproteins and lipids, including statherin, amylase, proline-rich peptides (PRPs)

Busscher HJ, van der Mei HC: Physico-chemical interactions in initial microbial adhesion and relevance for biofilm formation. Adv Dent Res 1997;11:24–32.

FUNCTIONS OF PELLICLE

- Protects the tissue from desiccation
- Acts as a receptor favoring bacterial attachment



STEP 2:TRANSPORT OF MICROORGANISMS AND REVERSIBLE ATTACHMENT

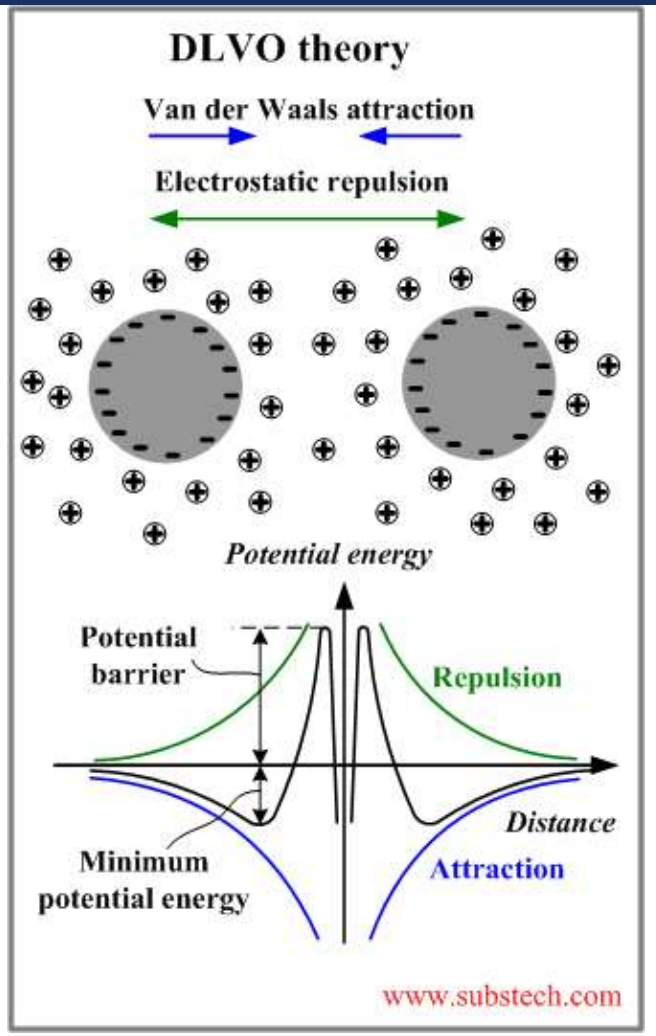
Microorganisms are transported to the surface by

- ✓ Brownian movement (40µm/hour)
- ✓ Sedimentation of bacteria
- ✓ Chemotactic action

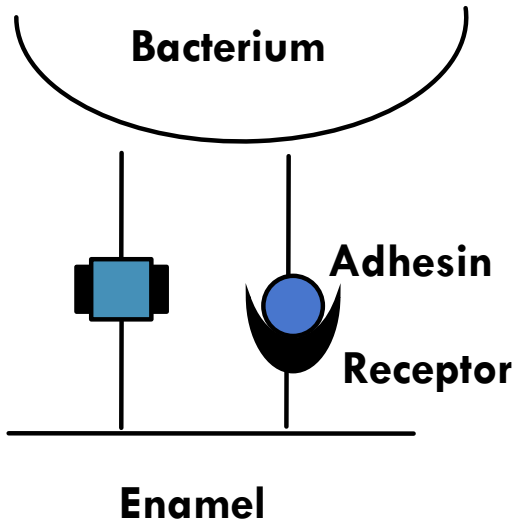
As the cell approaches the pellicle-coated surface, due to long range relatively weak and short range physiochemical forces it gets reversibly attached to the tooth.

MECHANISM OF REVERSIBLE ATTACHMENT

- As a particle approaches a surface it experiences a weak Vander Waals attraction induced by the fluctuating dipoles within the molecules of the two approaching surfaces.
- This attraction increases as the particle moves closer to the substratum.
- A repulsive force is encountered if the surfaces continue to approach each other, due to the overlap of the electrical double layers.



STEP 2 : PIONEER MICROBIAL COLONIZERS AND IRREVERSIBLE ATTACHMENT (ADHESIN–RECEPTOR INTERACTIONS)



Attachment of bacteria

- Established by specific interaction
- Ionic bond, covalent bond, hydrogen bond
- Bonding is mediated by specific extracellular protein **Adhesin** and its receptor in pellicle

VARIOUS BACTERIAL ADHESIN AND THEIR RECEPTOR IN ACQUIRED PELLICLE

Bacterium	Adhesin	Receptor
Streptococcus spp.	Antigen 1/11	Salivary Agglutinin
Mutans streptococci	Glucan binding protein	Glucan
Streptococcus parasanguinis	35 kda lipoprotein	Fibrin, Pellicle
Actinomyces naeslundii	Type 1 fimbriae	Proline-rich Proteins
Porphyromonas gingivalis	150 kda protein	Fibrinogen
Prevotella loescheii	70 kda lectin	Galactose
Fusobacterium nucleatum Coaggregation with *P. Gingivalis*	42 kda protein	

STEP 3 :COLONIZATION OF BACTERIA BY CO-AGGREGATION

- Firmly attached bacteria grow and form new inter-bacterial interaction
- The ability of different species and genera of plaque microorganisms to adhere to one another by a process known as ***co-aggregation.***
- Co aggregation is cell to cell recognition of genetically distinct partner cell type
- 18 genera in oral cavity have shown some form of Co-aggregation
- Process occurs by specific stereo chemical interaction of protein and carbohydrate molecules located on cell surface of bacteria

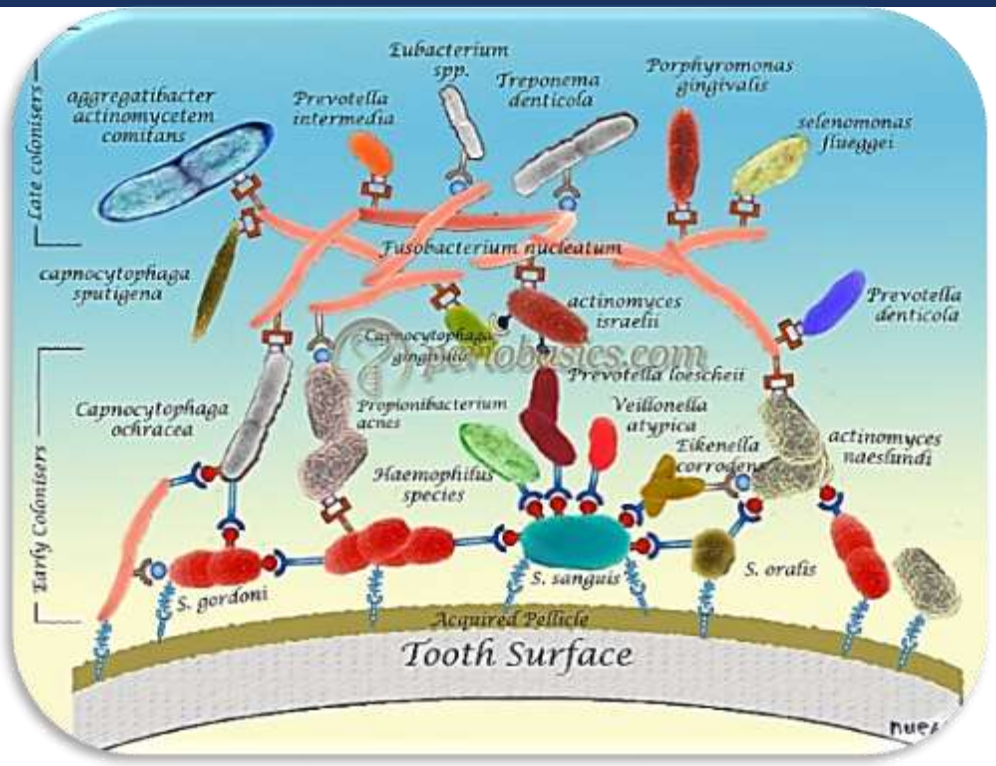
(Kolenbrander PE ,1993).

CO-AGGREGATION

- Early plaque accumulation is facilitated by *intrageneric* co-aggregation.
- The subsequent development of dental plaque will involve further *intergeneric co-aggregation* between other genera and the primary colonies for example co-aggregation can occur between:

- ***Gram-positive species:*** Ex: *S. Sanguis* and *Actinomyces Sp.*
- ***Gram negative species:*** Ex: *P. Melaninogenica* and *F.nucleatum*
- ***Gram positive and gram negative species:***
  - Ex: *Streptococcus* and *Prevotella Species*,
  - *Actinomyces* Species with *Capnocytophaga* sp., *F.nucleatum*, *E.corrodens*, *Veillonella* Species , *P. gingivalis* .

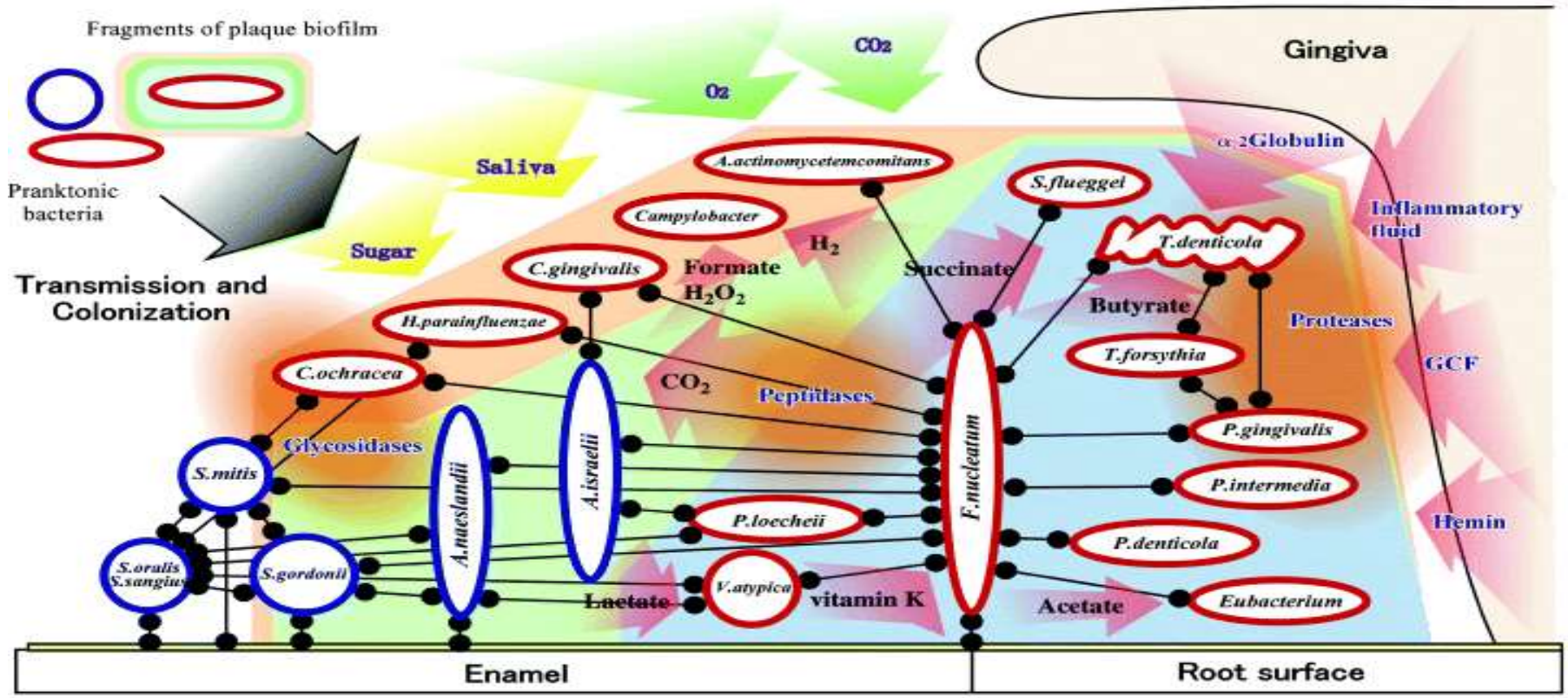
CO-AGGREGATION



- ***Co-aggregation*** often involves ***lectins***, these carbohydrate-binding proteins interact with the complementary carbohydrate containing receptors on another cell

CO-AGGREGATION OF BACTERIA

Coaggegation is mediated by lectin like adhesin and inhibited by lactose and galactose



Early colonizer ⇨ Secondary colonizer ⇨ Late colonizer : Microbial succession

(Gram ⊕ cocci) (Gram ⊕ ⊖ rod) (Gram ⊖ rod/ motile) (Dominant microorganisms)

Aerobic ⇨ Capnophilic / Facultative anaerobic ⇨ Anaerobic : Environment modification

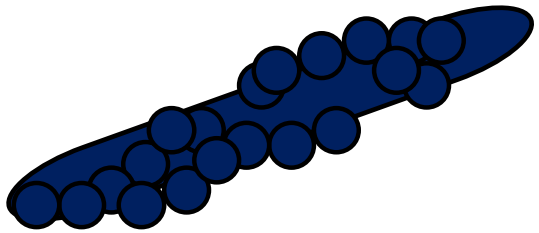
Saliva (saccharolytic) ⇨ GCF (proteolytic) : Nutrition supply

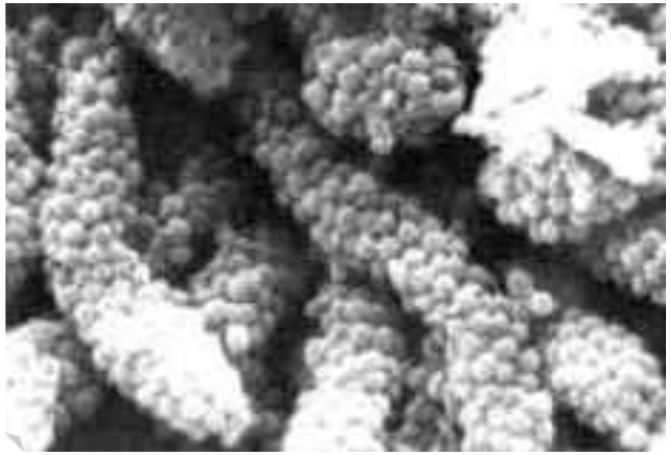
STEP 4 : CLIMAX COMMUNITY

- The interaction between the microbial and non-microbial components of an ecosystem ultimately leads to a form of stabilization in which microbial and non-microbial forms exist in harmony and equilibrium with their environment. This is the ***climax community.***

- This remains reasonably stable over time and reflects a dynamic situation in which cells are dying and being replaced.

CLIMAX COMMUNITY

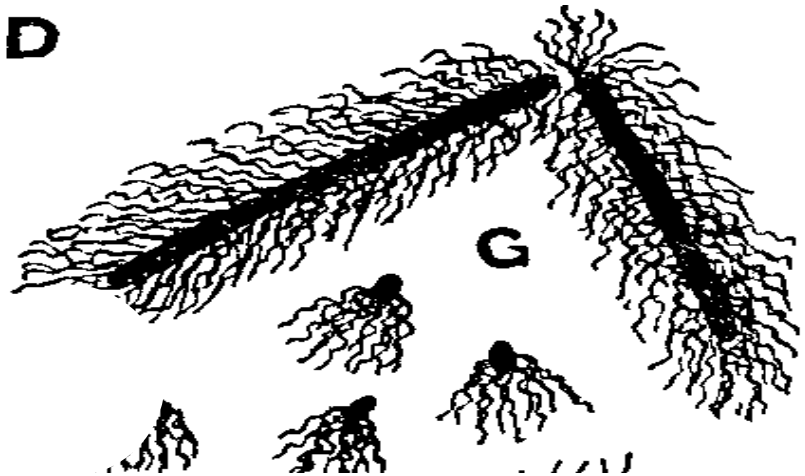


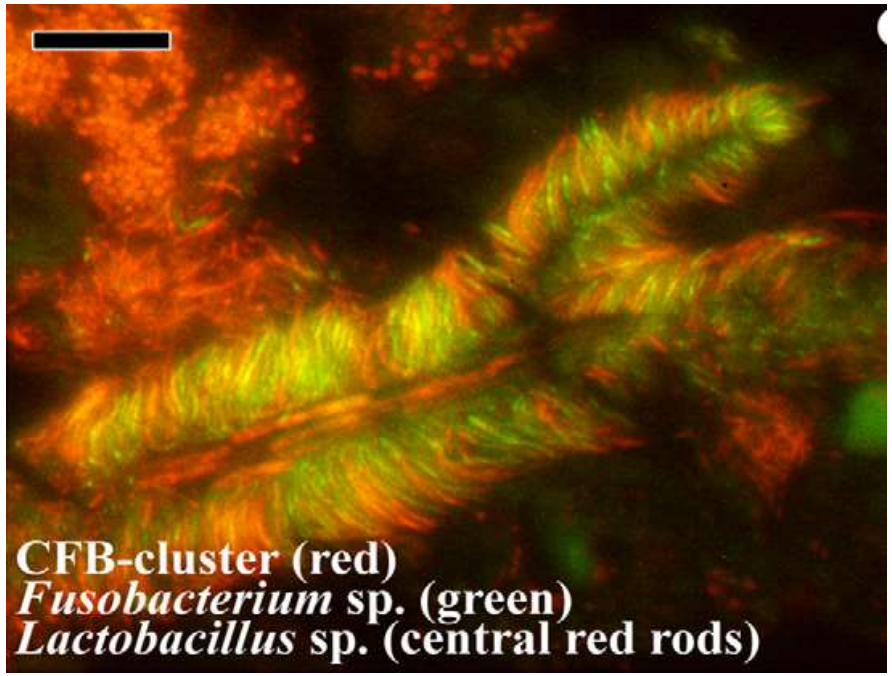


Longstanding supragingival plaque near gingival margin shows

<u>Corncob structure:</u>
Central G-ve rod outer G+ve cocci cluster

CLIMAX COMMUNITY





Sub-gingival tissue associated plaque shows

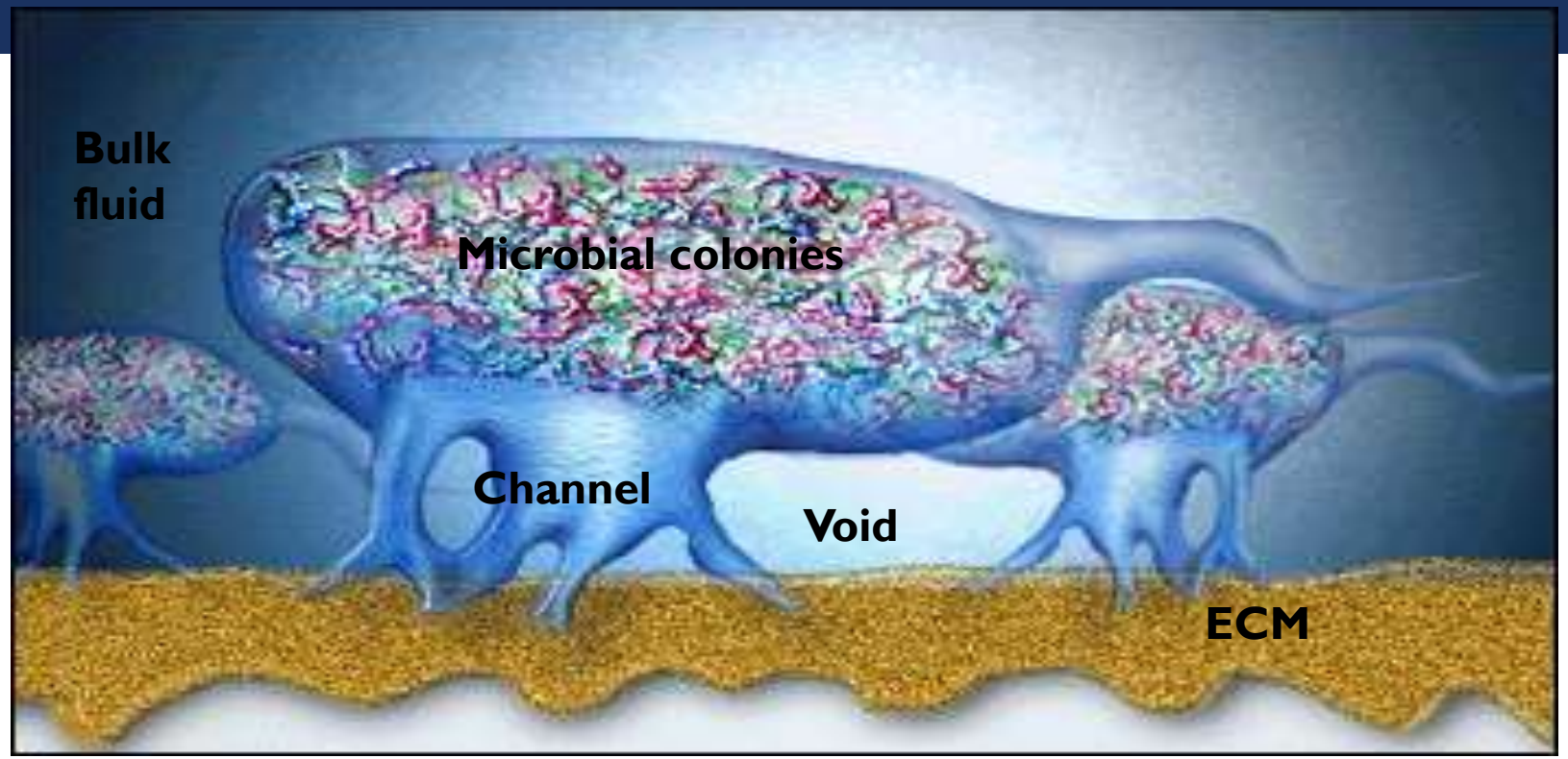
Test tube brush structure

Flagellated bacteria with large central flagella and numerous short flagella embedded in matrix

Test-tube brush with Lactobacillus sp. (red rods) as central structures. F. nucleatum (green) and Bacteroides cluster filaments radiating from the central structures.

STRUCTURE OF BIOFILM

STRUCTURE OF BIOFILM



- Biofilm is 3 dimensional structure made out of exopolymer matrix, embedded microbial communities, voids and channels for bulk fluids. It is tenaciously adherent to the tooth surface.

COMPOSITION

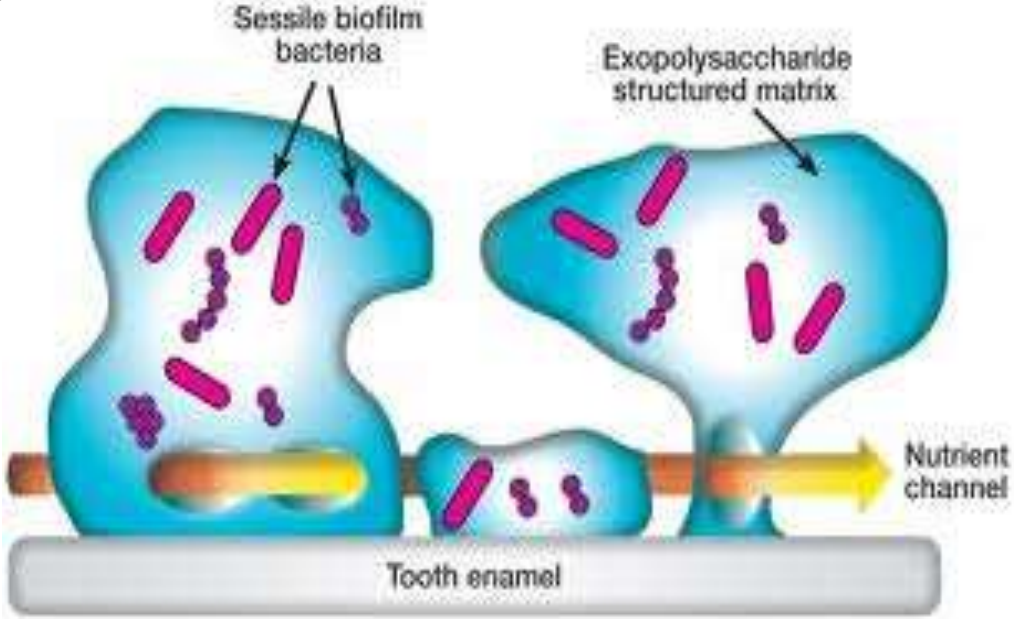
Dental plaque

- 80% Water
- 20% Solids
  - 35% Cellular(Bacteria) Dry weight
  - 65% Extracellular matrix Wet/Aqueous weight

Extracellular
Polysaccharide(EPS)
Lipids
Glycoproteins
Gingival exudate
Degenerated bacteria

EXOPOLYSACCHARIDES-BACKBONE OF BIOFILM

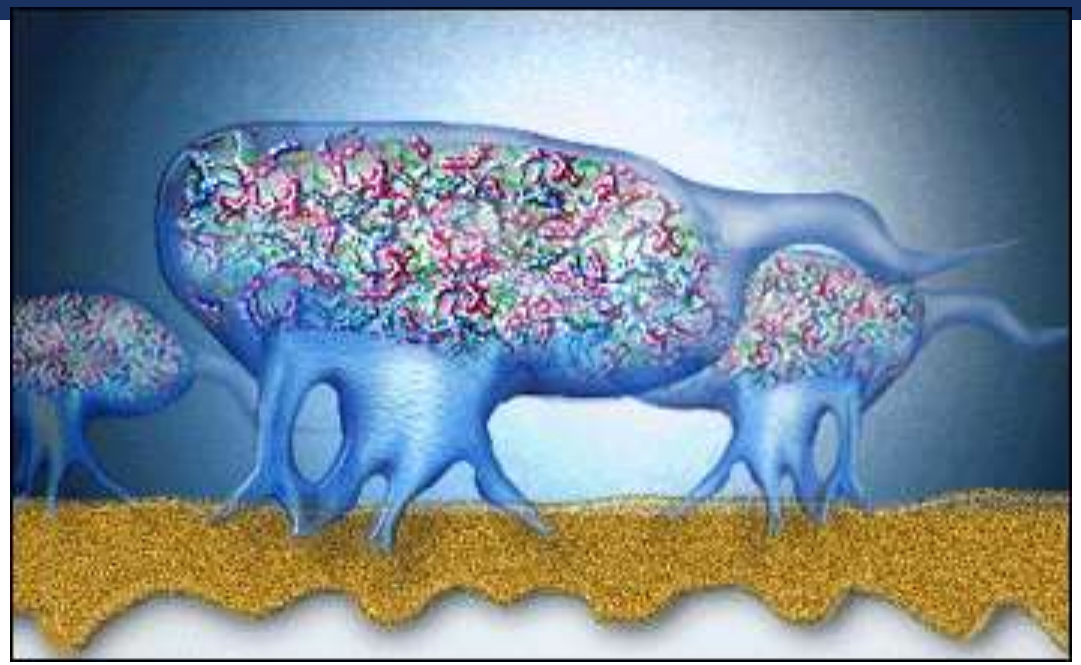
- Major component of biofilm is a mixture of exopolysaccharide, proteins, salts and cell material
- It is produced by bacteria
- It forms 50-95% of dry weight of biofilm



FUNCTIONS OF EXOPOLYSACCHARIDE MATRIX

- Maintains the *integrity* of the biofilm as well as preventing *desiccation* & attack by the harmful agents.
- Bind essential *nutrients* such as cations to create a local nutritionally rich environment favoring specific microorganisms.
- They can be *degraded* & *utilized* by bacteria within the biofilm.
- Act as a *buffer* & assist in the *retention* of extra cellular enzymes (& their substrates) enhancing substrate utilization by bacterial cells.

WATER CHANNELS



- The dental plaque biofilm is heterogeneous in structure, with a clear evidence of fluid filled channels running through the plaque mass

Costerton, Wood, Marsh PD

- Primitive circulatory system are the nutrient channels for bacterial colonization

PROPERTIES OF BIOFILM

PROPERTIES OF BIOFILM

- Biofilms are fascinating structures
- Every microbial biofilm community is unique
- Biofilm architecture is heterogeneous both in space and time, constantly changing because of external and internal processes
- Ranges from a patchy monolayer of cells to a film several layers thick

PROPERTIES OF BIOFILM

- Cooperating community of various types of microorganisms
- Microorganisms are arranged in micro colonies
- Micro colonies are surrounded by protective matrix
- Within the micro colonies are differing environments
- Microorganisms have primitive communication system
- Microorganisms in biofilm are resistant to antibiotics, antimicrobials, and host response.

- Surface microorganisms are more susceptible to detachment, a characteristic that facilitates travel to form new biofilm colonies on nearby oral structures and tissues

- Cell-to-cell signalling has recently been demonstrated to play a role in cell attachment and detachment from biofilms.

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PHYSIOLOGICAL HETEROGENEITY WITHIN BIOFILMS

- Cells of the same microbial species can exhibit extensively different physiologic status in a biofilm even though separated by as little as 10 microns.
- Bacterial cells, as indicated by presence of DNA is detected throughout the biofilms, but protein synthesis, respiratory activity and RNA are detected primarily in outer layers.
- pH can vary quite remarkably over short distances

- Measurement of oxygen and other gases have demonstrated that certain micro-colonies are completely anaerobic even though composed of single species.

- Number of metal ions can differ sufficiently in different regions of a biofilm so that differences in ion concentration can produce measurable potential differences.

- Bacterial cells within biofilm can produce enzymes such as β-lactamase against antibiotics or catalases or super oxide dismutases by phagocytes producing an almost impregnable line of defense.

Jan Lindhe

Microbial Interactions and communication

- In recent years there has been a paradigm shift in our understanding of the unicellular bacterial world from the perspective that bacterial cells are non-cooperative to one which incorporates social interactions and multicellular behaviour

- Bacteria are clearly capable of complex patterns of co-operative behaviour that result from the coordination of the activities of individual cells.

- Some of the functions of biofilm are dependent on the abilities of the bacteria & micro colonies within the biofilm to communicate with one another.

These interactions can be of various types:

(i) competition between bacteria,

(ii) synergistic interactions which may stimulate the growth or survival of one or more residents,

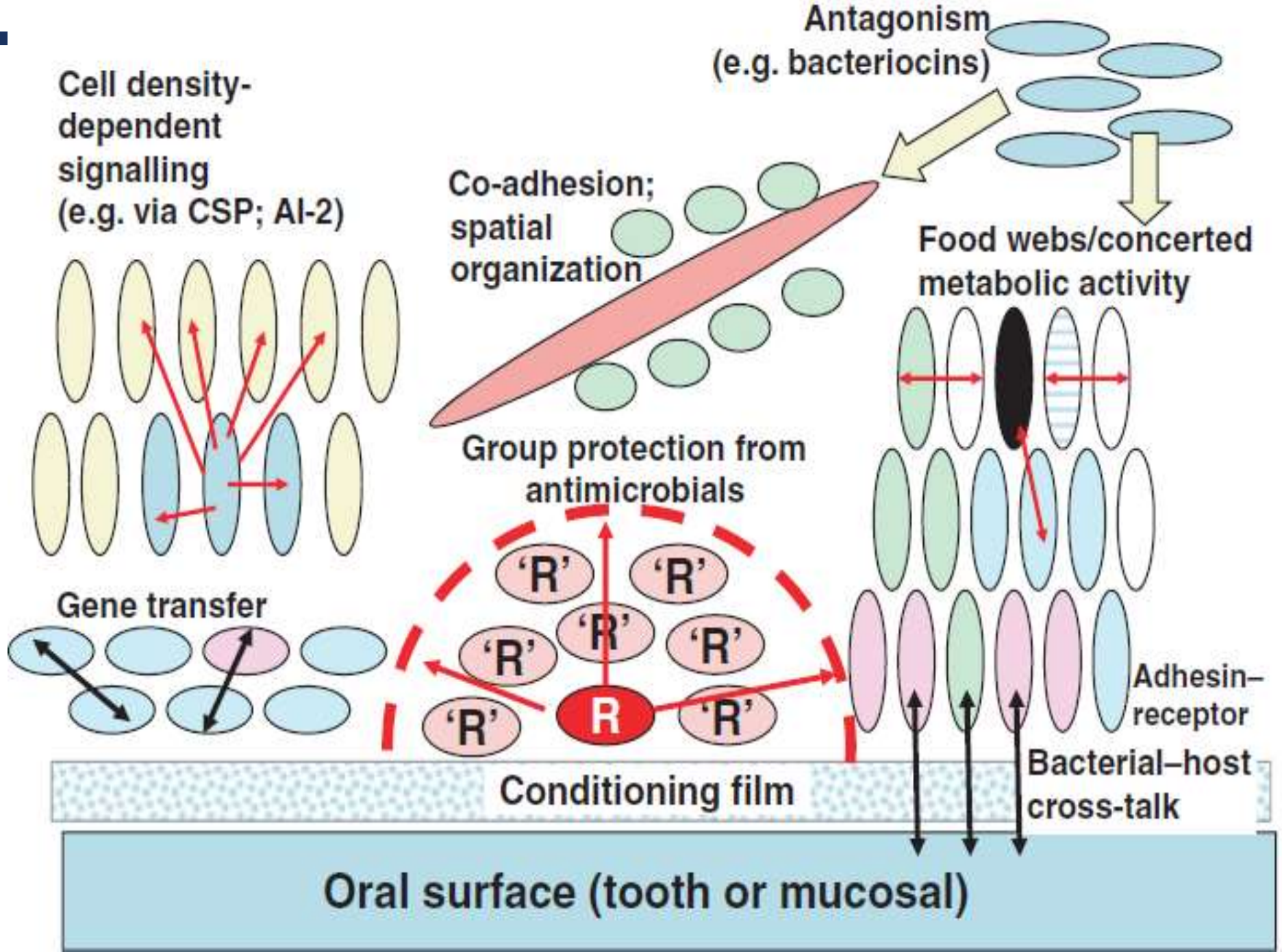
(iii) production of an antagonist by one resident which inhibits the growth of another,

(iv) neutralization of a virulence factor produced by one organism by another resident, and

(v) interference in the growth-dependent signaling mechanisms of one organism by another.

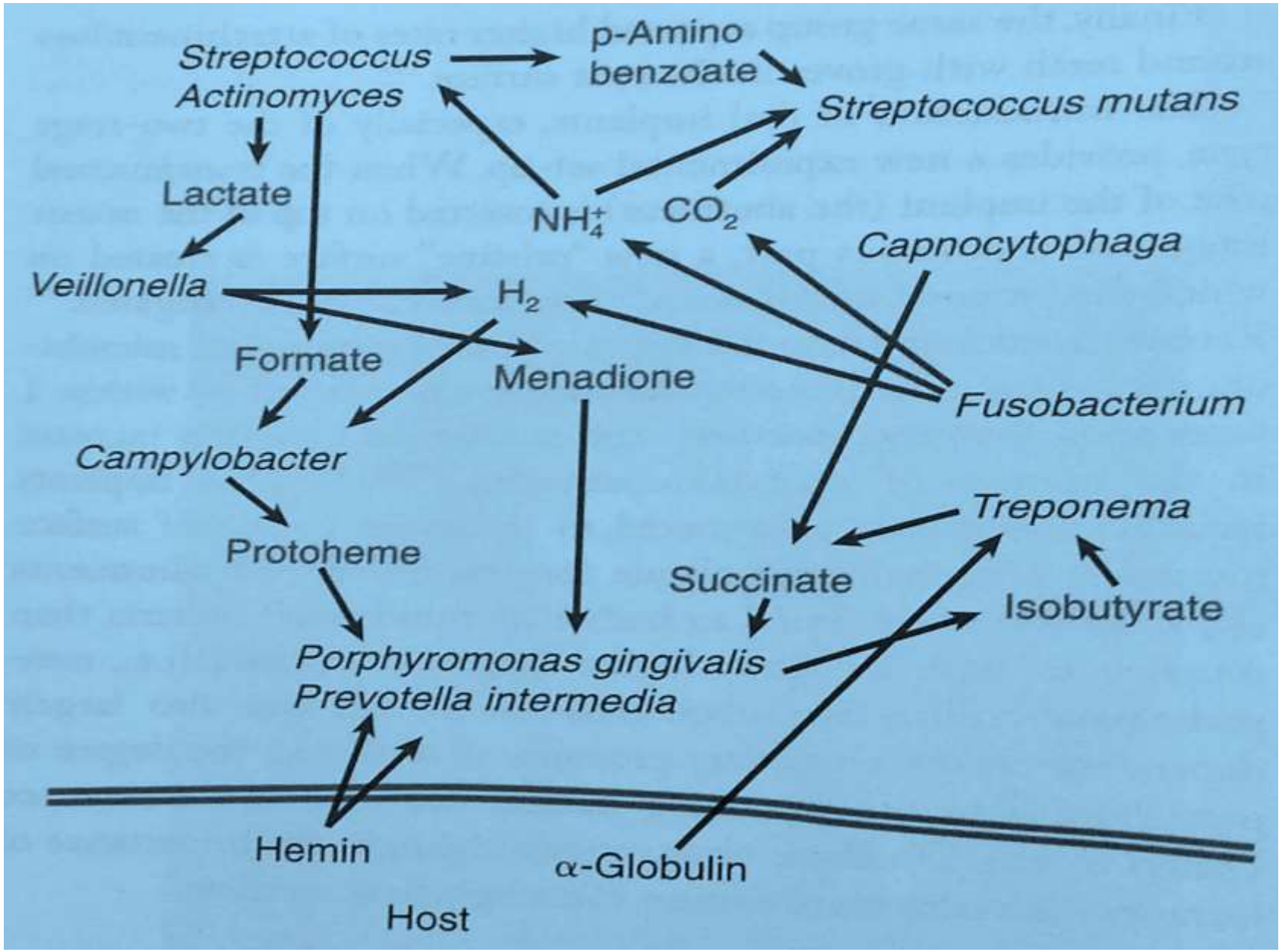
- These interactions occur at several levels, which includes:

  - Metabolic exchange,
  - Small-signal-molecule-mediated communication and
  - Exchange of genetic material
  - Physical contact.


Antagonism
(e.g. bacteriocins)
Cell density-
dependent
signalling
(e.g. via CSP; AI-2)
Co-adhesion;
spatial
organization
Food webs/concerted
metabolic activity
Group protection from
antimicrobials
'R'
'R'
'R'
'R'
'R'
'R'
R
'R'
Gene transfer
Adhesin–
receptor
Bacterial–host
cross-talk
Conditioning film
Oral surface (tooth or mucosal)

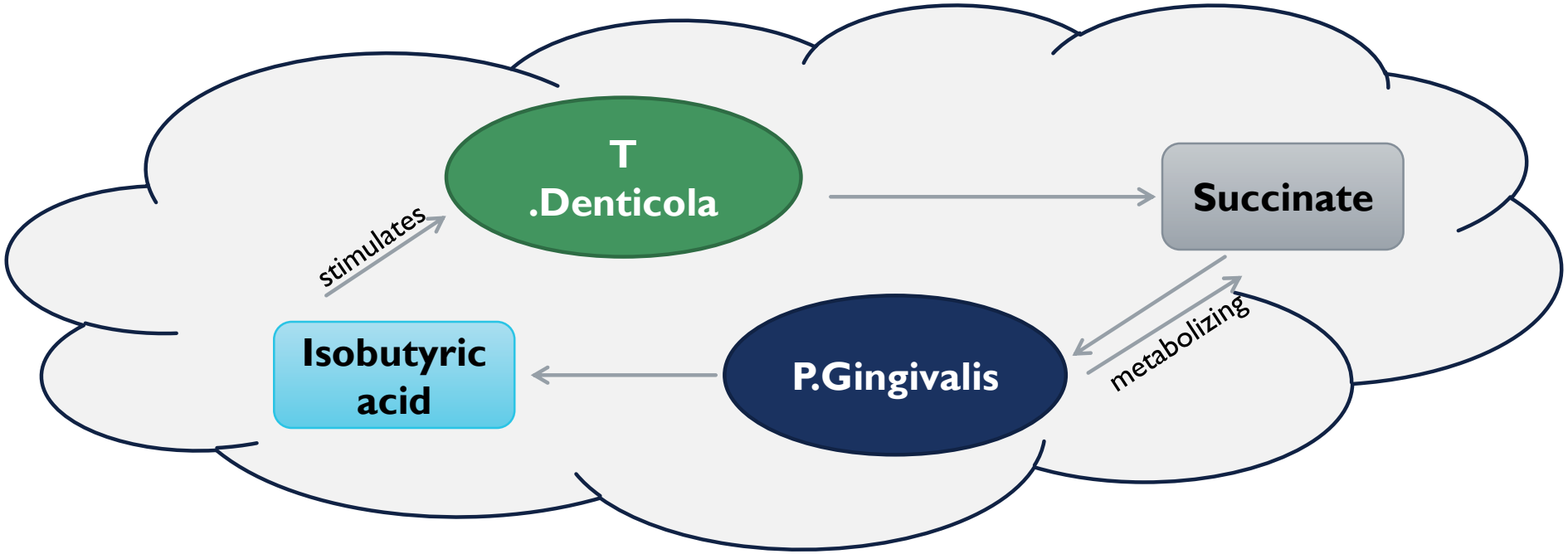
METABOLIC COOPERATION

- One important factor in determining the bacterial composition of a biofilm is clearly the availability of nutrients.
- Organisms that have adapted to particular environments such as the oral cavity have evolved metabolic pathways to efficiently utilize the available nutrients in each specific ecological niche.
- These nutrients are available from the periodic intake of food, saliva, and nutrients provided by other organisms as well as polysaccharides present in dental plaque


Streptococcus
Actinomyces
p-Amino
benzoate
Streptococcus mutans
Lactate
NH_4^+
CO_2
Capnocytophaga
Veillonella
H_2
Formate
Menadione
Fusobacterium
Campylobacter
Treponema
Protoheme
Succinate
Isobutyrate
Porphyromonas gingivalis
Prevotella intermedia
Hemin
α-Globulin
Host

- *Porphyromonas gingivalis*, one of the major etiological agents of periodontal disease, often coexists with other periodontopathic bacteria, due to nutritional interdependence.

- Grenier and Maryrand demonstrated crossfeeding between *P. gingivalis* and *T. denticola*



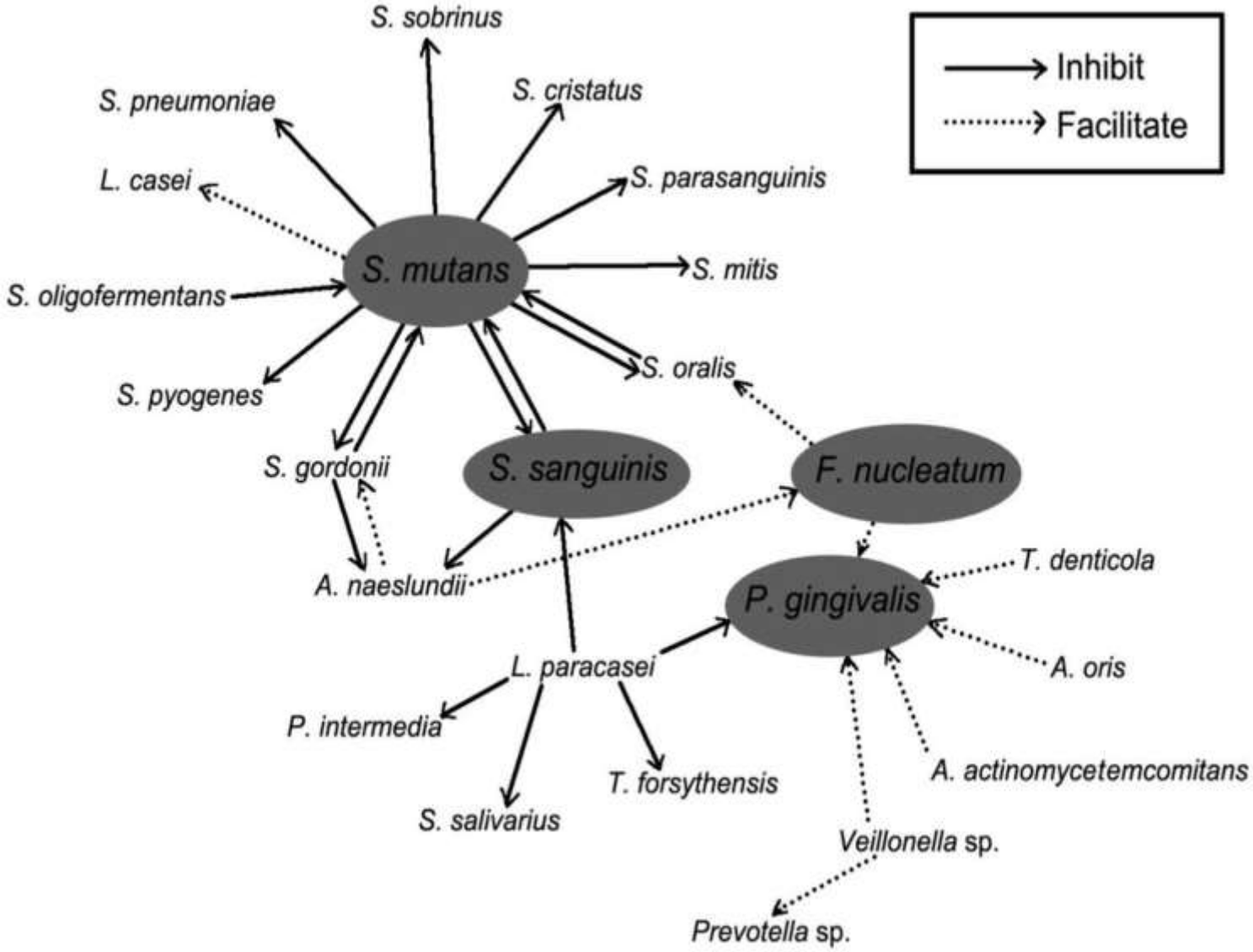
Grenier.D, and D. Maryrand. Infect. Immun 1986 53:616–620.

- Synergistic or mutualistic interactions between two organisms in biofilms that appear to be dependent upon saliva have been demonstrated for *Actinomyces naeslundii* and *Streptococcus Oralis*

- Either organism alone is a poor colonizer of saliva-coated surfaces, but together they form extensive biofilms on these same surfaces. This appears to result from the combined metabolic activities of the two organisms in metabolizing salivary components.

Palmer, R. J., Jr., K. Kazmerzak, M. C. Hansen, and P. E. Kolenbrander. Infect. Immun. 2001; 169:5794–5804.

SYNERGY AND ANTAGONISM



Small-Signal-Molecule-Mediated Communication

Yung-Hua Li and Xiaolin Tian
Sensors 2012, 12, 2519-2538

- Many bacteria have been found to regulate diverse physiological processes and group activities in biofilm through a mechanism called **quorum sensing**.

- It is defined as the **cell density** dependent regulation of **gene expression** in response to soluble signals called autoinducers

- Quorum sensing in bacteria “involves the regulation of expression of specific genes through the accumulation of signaling compounds that mediate inter cellular communication.”

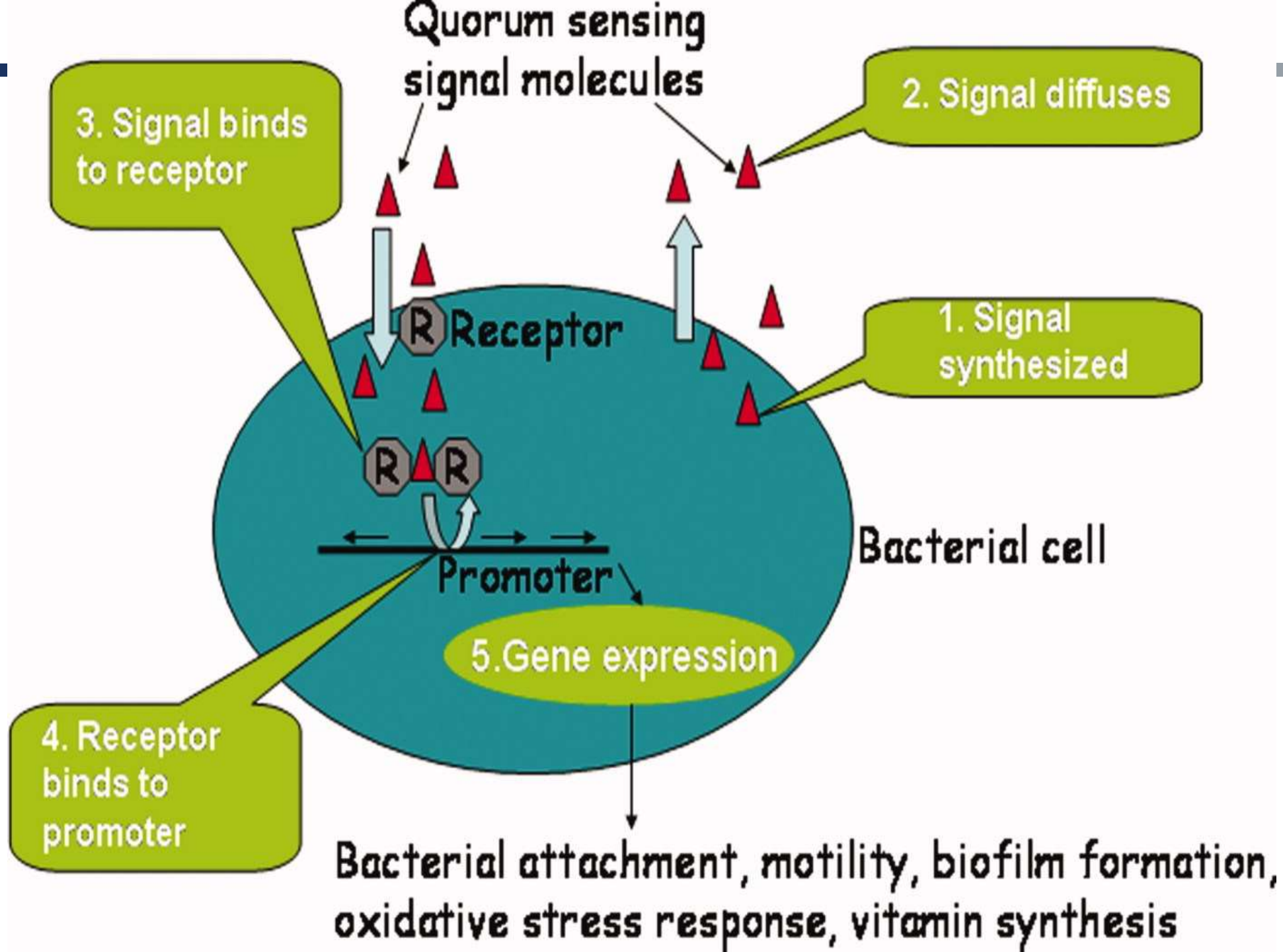
(Bassler 1999)

- In QS bacterial cells produce, detect and respond to small diffusible signal molecule

- The term quorum is used because a *number of microorganisms must be present for the signal to be felt and populations to respond.*

- This concentration is called *quorum density*.

- Quorum sensing was first described in the marine bioluminescent bacterium *Vibrio fischeri*


Quorum sensing signal molecules
2. Signal diffuses
3. Signal binds to receptor
R Receptor
1. Signal synthesized
R R
Promoter
Bacterial cell
5.Gene expression
4. Receptor binds to promoter
Bacterial attachment, motility, biofilm formation, oxidative stress response, vitamin synthesis

- In many bacteria, quorum sensing represents a central mechanism to regulate ***social activities***, allowing bacteria to reap benefits that would be unattainable to them as individual cells

- Quorum sensing-mediated social activities favor microbial interactions and are believed as major mechanisms to regulate population-level ***virulence*** of bacteria

- Quorum sensing relies upon the interaction of a small diffusible signal molecule with a sensor or transcriptional activator to initiate gene expression for coordinated activities

Types of Quorum sensing molecules

Autoinducer 1

- 1st detected in Vibrio fisheri - by Nealson *et al* 1979
- Chemically – N – Acyl Homoserine Lactone(AHL)
- Proteins involved are designated as
  - Lux I & Lux R
  - Lux I - Catalyses the synthesis of AHL
  - Lux R - transcriptional regulator
- Autoinducer-1 is not common in oral biofilm.
- It usually regulates gene expression in genetically identical cells

Paul Williams Microbiology (2007), 153, 3923–3938

Autoinducer 2

- First observed by Schauder et al 2001
- Collection of molecules formed from spontaneous rearrangement of 4,5 dihydroxy-2-3 pentanedione (DPP)
-
- Produced by both gram +ve & -ve organism
- Gene responsible for its production - *lux* S - protein
- It usually regulates gene expression in genetically different cells

Paul Williams Microbiology (2007), 153, 3923–3938

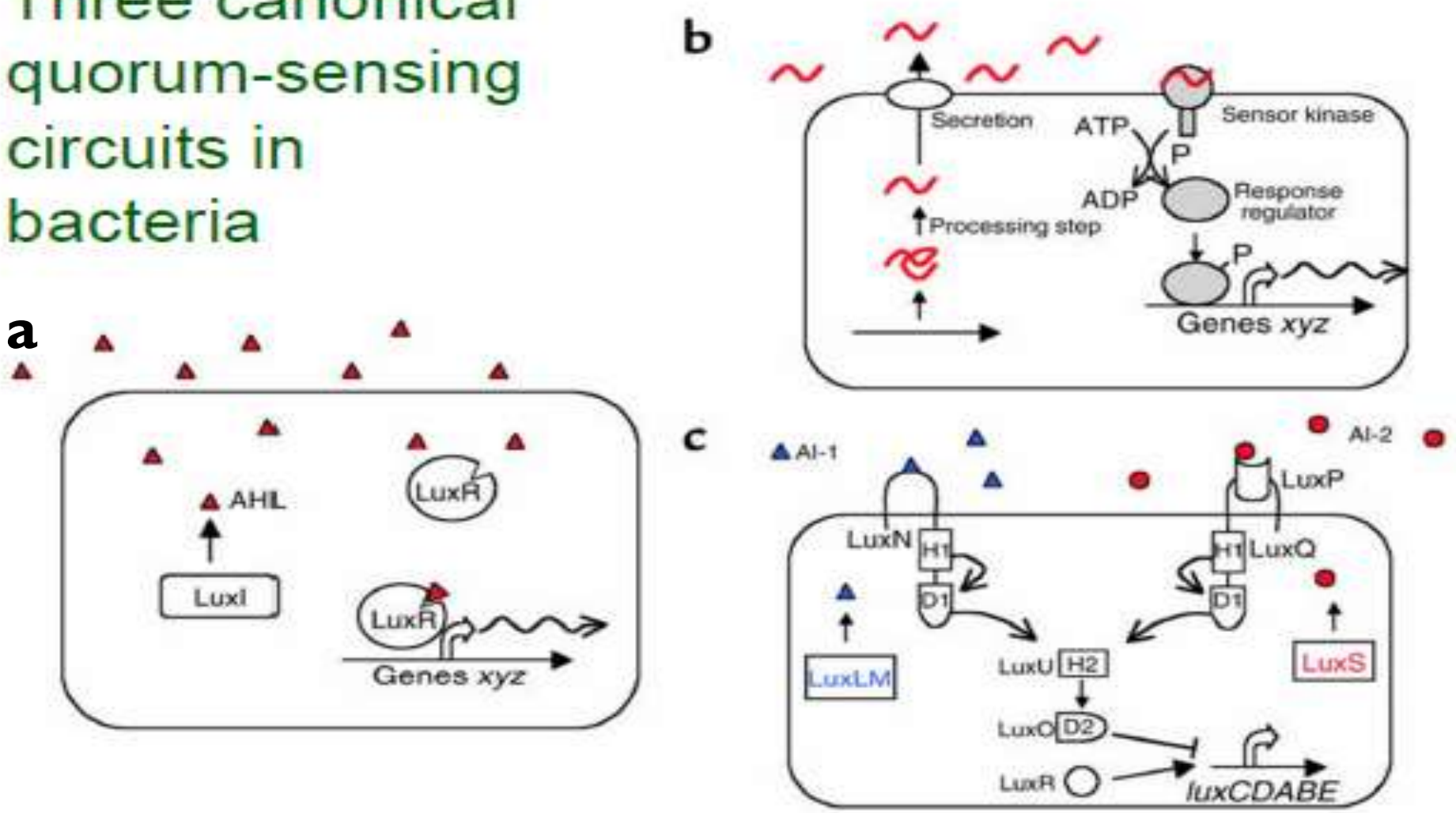
Competence-stimulating peptide (CSP)

- Competence-stimulating peptide (CSP) is a small soluble peptide having from 14-23 amino acid residues and is potentially produced by many species of streptococci.
- Implicated in bacteriocin production, virulence and biofilm formation.

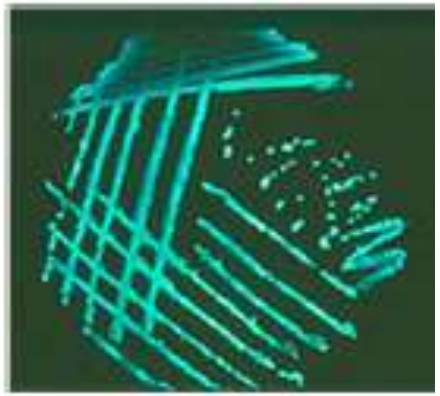
Paul Williams Microbiology (2007), 153, 3923–3938

Summary for Quorum Sensing Systems

Three canonical quorum-sensing circuits in bacteria



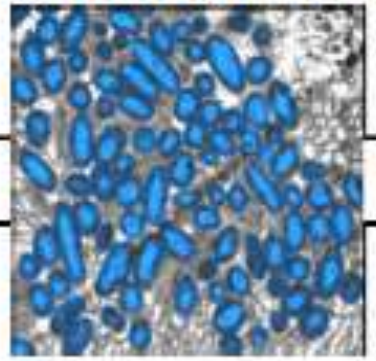
Multilingual bacteria



Vibrio harveyi

- **A bioluminescence Gram (-) marine bacterium ; free living in the ocean**

Communication system



AI-1 molecule

AI-2 molecule

Speaks language unique to V. harveyi

Speaks universal language used by many quorum-sensing bacteria

Microbial Gene Transfer

Gene Transfer

- Cells also communicate with one another in biofilms via **horizontal gene transfer.**
- Molecules such as CSP markedly increase the ability of recipient cells in biofilm to take up DNA *(Li et al 2002b)*.
- The high density of bacterial cells growing in biofilms facilitates exchange of genetic information between cells of the same species & across species or even genera.
- ***Conjugation***, ***transformation***, ***plasmid transfer*** & ***transposon*** have all been shown to occur in naturally occurring or in vitro prepared mixed species biofilms.

- The transfer of conjugative transposons encoding tetracycline between streptococci in model biofilms has been demonstrated

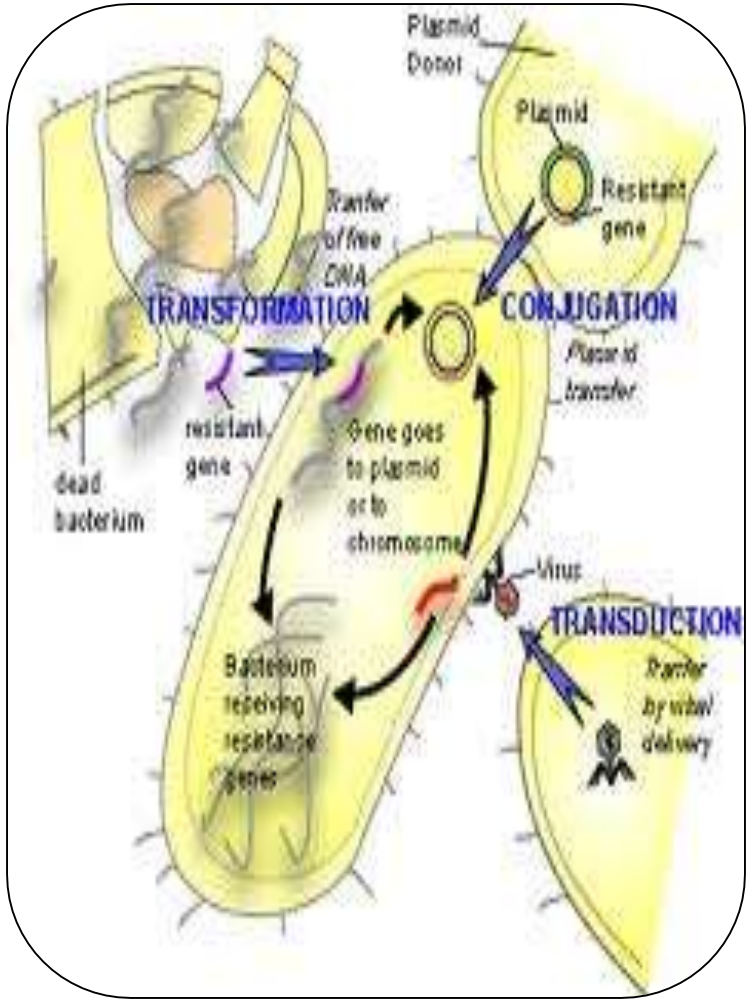
  (Roberts et al 2001)

- Similar evidence suggests sharing of genes responsible for penicillin binding proteins among commensal & pathogenic Neisseria

  (Bowler et al 1994)

- These finding suggests that plaque can function as a **genotypic reservoir** by harboring transferable mobile elements & genes.

Genetic exchange



Conjugation

Bacterium —plasmid→ Bacterium

Transduction

Bacterium —bacteriophage→ Bacterium

Transformation

DNA outside the cell is fragmented and combined with bacterial DNA

Mobile Genetic Elements

- *Transposons* – elements capable of excision from the chromosome of the donor genome, transfer to recipient cell and get integrates its genome.

- *Integron* – gene cassette system – mechanism that allows bacteria to accumulate diverse genes at a common locus, useful in acquiring antibiotic resistance

- *Genomic islands* – regions of genome acquired horizontally.

- Combination of these.

Anti-microbial Resistance

Antibiotic Resistance of Organisms in Biofilm

- Organisms growing in biofilms are more resistant to antibiotics than same species growing in planktonic state.

  (Gilbert et al. 1997, 2002, Ceri et al. 1999, Stewart & Costerton 2001)

- Slower rate of growth of bacterial species in the biofilms, which makes them less susceptible antibiotics.

- Cells deep in biofilm experience different conditions such as H+ ion concentration or redox potential than cells at the periphery of the biofilm or cells growing planktonically.

- Growth rates of these deeper cells are decreased, allowing them to survive better than faster growing cells at the periphery when exposed to antimicrobial agents.

- Slower growing bacteria over express "*non specific defense mechanisms*" including shock proteins & multi-drug efflux pumps (arcAB) & demonstrate increased exopolymer synthesis.

- The ability of the matrix to act, as a *physical barrier*.

- Extra cellular enzymes such as *β-Lactamases*, *formaldehyde lyase* & *formaldehyde dehydrogenase* may become trapped & inactivating susceptible, typically positively charged, hydrophilic antibiotics.

- Cells growing within a biofilm express genes that are not observed in the same cells grown in planktonic state & they can retain this resistance for sometime after being released from the biofilm.

- The presence of glycocalyx, a slower growth rate & development of a biofilm phenotype cannot provide a total explanation for the phenomenon of antibiotic resistance.

- Recently the notion of a subpopulation of cells within a biofilm, that are "***super resistant***" was proposed.

- Such cells could explain remarkably elevated levels of resistance to certain antibiotics.

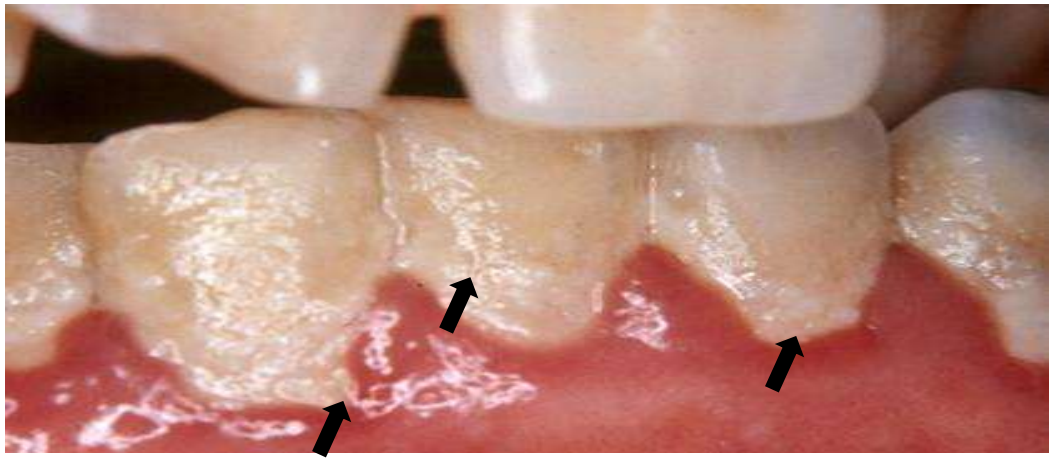
- Broun et al examined the contribution of ***multi drug resistance pumps*** to antibiotic resistance of organisms growth in biofilms.

- These “pumps” can extrude chemically unrelated antimicrobial agents from the cell.

- Since extrusion places the antibiotics outside the outer membrane, the process offers protection against antibiotics that target cell wall synthesis.

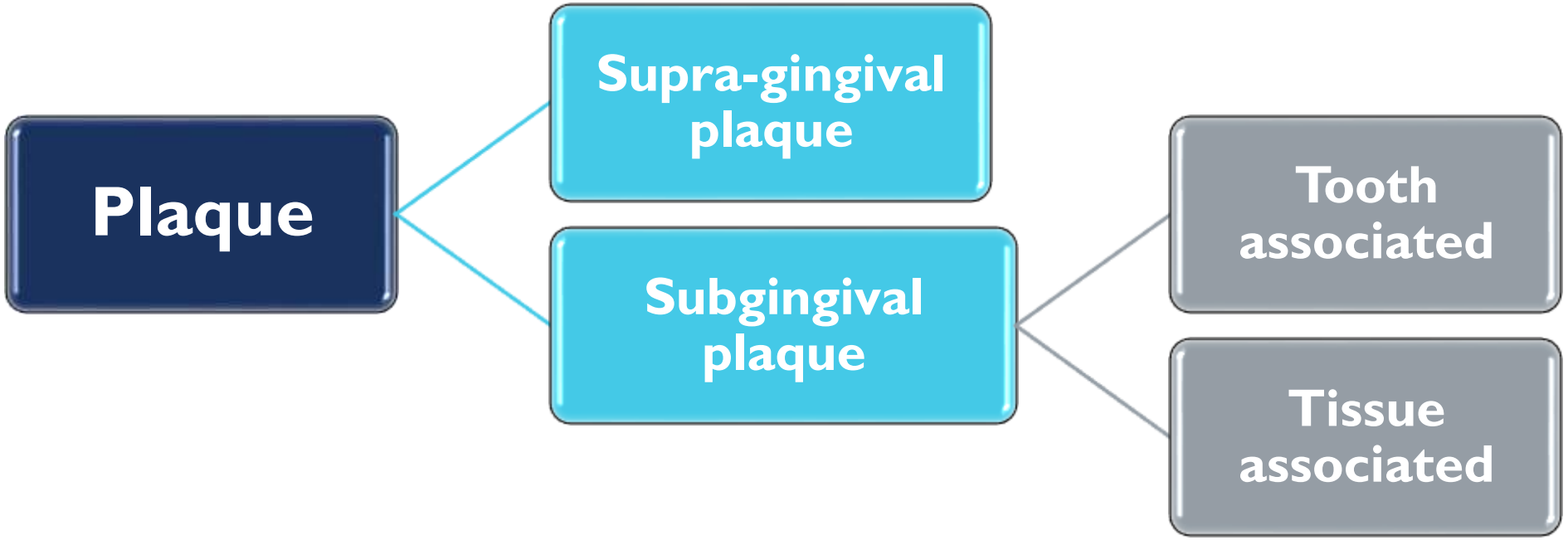
Dental plaque
A tooth associated biofilm

MACROSCOPIC APPEARANCE OF PLAQUE



- Whitish, Grayish/ yellowish color , has a globular appearance
- Readily visible on the tooth surface after 1-2 days.
- Accumulates in the gingival third and in pits, cracks, fissures, overhanging restorations and around mal aligned tooth.
- Removed by movement of tissues and food materials.

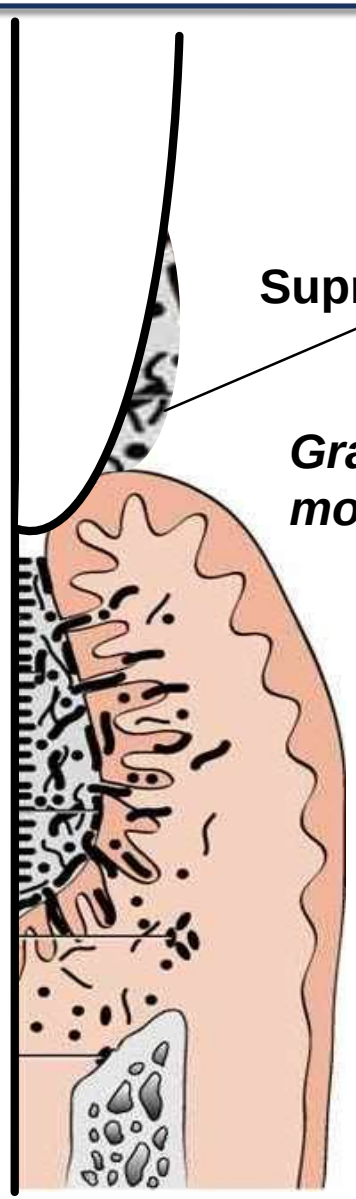
CLASSIFICATION OF DENTAL PLAQUE



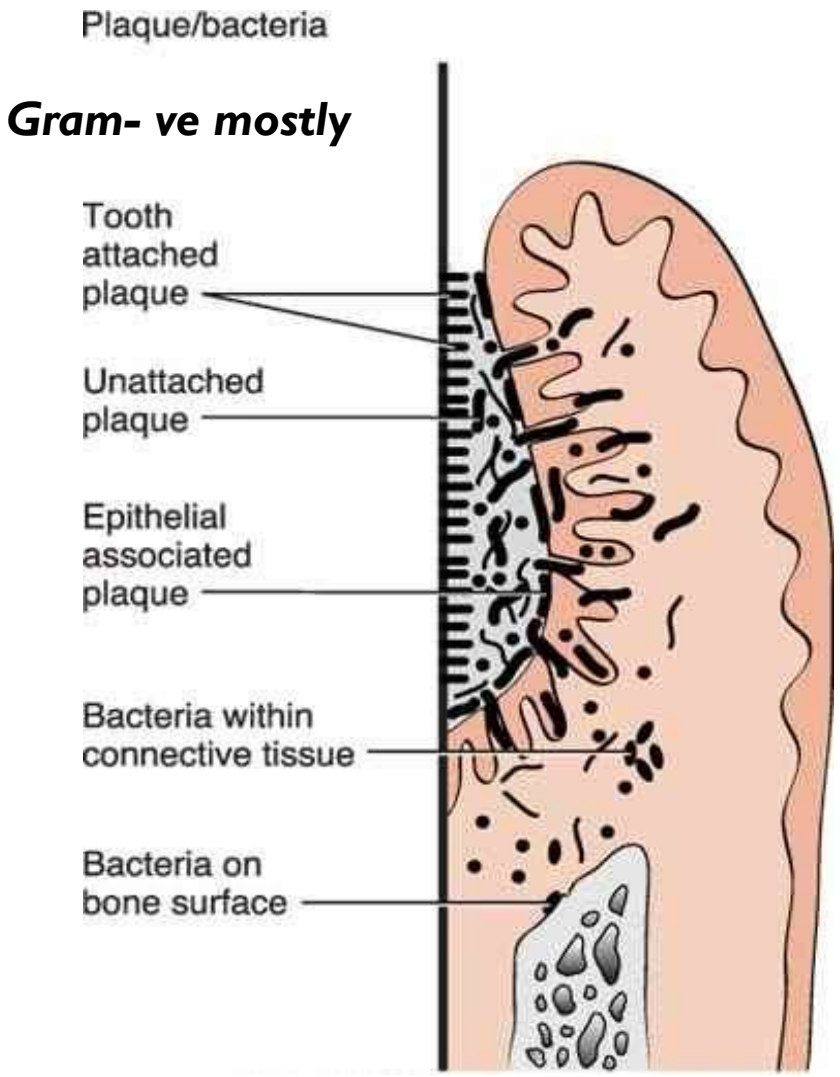
DIFFERENCES

	Supra gingival plaque	Sub gingival plaque
Matrix	50% matrix	Little or no matrix
Flora	Mostly G+ve	Mostly G-ve
Mobile bacteria	Few	Common
Anaerobic/aerobic	Aerobic unless thick	Highly anaerobic areas
Metabolism	Carbohydrates	Mostly proteins

Supragingival Plaque



Subgingival Plaque



CHARACTERISTICS OF SUBGINGIVAL PLAQUE

Tooth associated	Epithelium associated
G+ve bacteria predominates	Both G+ve and G – ve
Does not extend to junctional epithelium	Extends to junctional epithelium
May penetrate cementum	May penetrate cementum and connective tissue
Associated with calculus formation and root caries	Associated with gingivitis and periodontitis

MICROBIAL COMPLEXES IN SUBGINGIVAL PLAQUE

- The association of bacteria within mixed biofilms is not random, rather there are specific associations among bacterial species.

- ***Socransky et al 1998*** examined over 13,000 subgingival plaque samples from 185 adult subjects and used cluster analysis and community ordination techniques to demonstrate the presence of species microbial groups with dental plaque.

Socransky SS, Haffajee AD, Microbial complexes in subgingival plaque. J Clin Periodontol 1998: 25: 134–144

- Six closely associated groups of bacterial species were recognized.

- These included the *Actinomyces*, a yellow complex consisting of members of the genus *streptococcus* a purple complex consisting of *V. parvula* and *Actinomyces odontolyticus*.

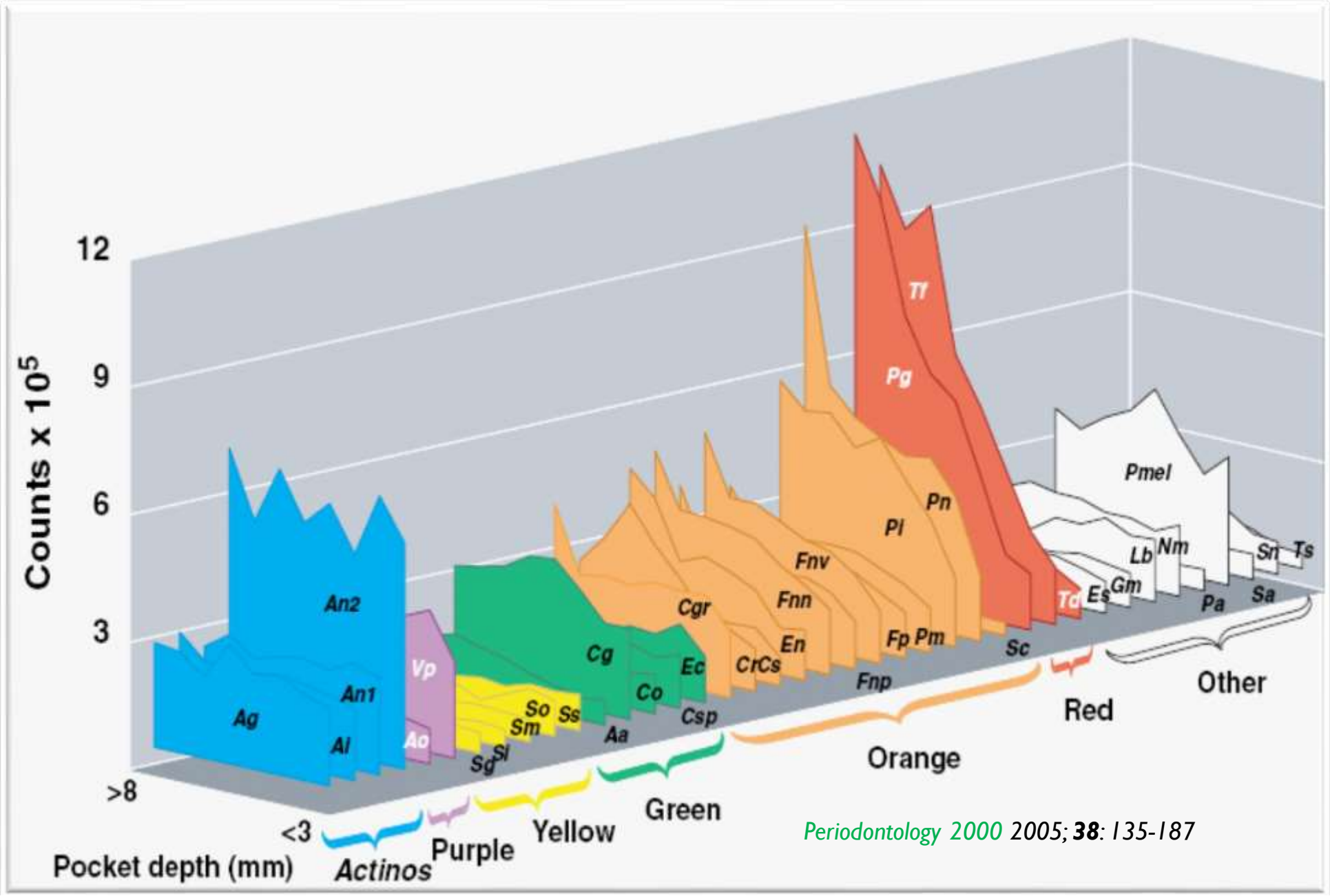
- These groups of species are early colonizers of tooth surfaces whose growth usually precedes the multiplication of the predominantly, gram-negative orange and red complexes.

Socransky SS, Haffajee AD, Microbial complexes in subgingival plaque. J Clin Periodontol 1998: 25: 134–144

Distribution of different complexes in subgingival plaque sample

Kigure et al J Periodontal Res 1995: 30: 332–341.

Pocket Depth and Microbial Composition



Periodontology 2000 2005; **38**: 135-187

BIOFILM ON DENTAL IMPLANT

BIOFILM ON DENTAL IMPLANT

- The sequence of microbial colonization on dental implants and biofilm formation is similar to that of teeth.

- Exposure of different implant materials in vivo to the oral environment showed that streptococci were the predominant initial colonizing microbes

- Streptococci were predominant after 4 hours, and anaerobes increased at 48 hours; this was common to all the implant materials studied. These studies indicate that the surface properties of implants influenced early bacterial adhesion but not the bacterial flora or plaque maturation.

- Bacterial colonization occurred within 30 minutes after implant placement, and early colonization patterns differed between implant and tooth surfaces based on the colonizing bacterial species.

- ***Mombelli et al. 1987, 1995*** in cross sectional and longitudinal studies concluded that structure of peri-implant plaque deposits resembled that encountered in the sub-gingival environment.

Peri-implant infection

Adhering plaque closely resembles the structure of subgingival microbiota encountered in advanced periodontitis

ROLE OF TOOTH ASSOCIATED BIOFILM IN PERIODONTAL HEALTH AND DISEASE

ROLE OF DENTAL PLAQUE IN HEALTH

- Dental plaque is part of the natural resident micro flora of the human body.

- The resident micro flora also reduces the risk of infection by acting as a barrier to colonization by exogenous (and often pathogenic) species (a phenomenon termed ‘colonization resistance’).

Mechanisms contributing to this colonization resistance include

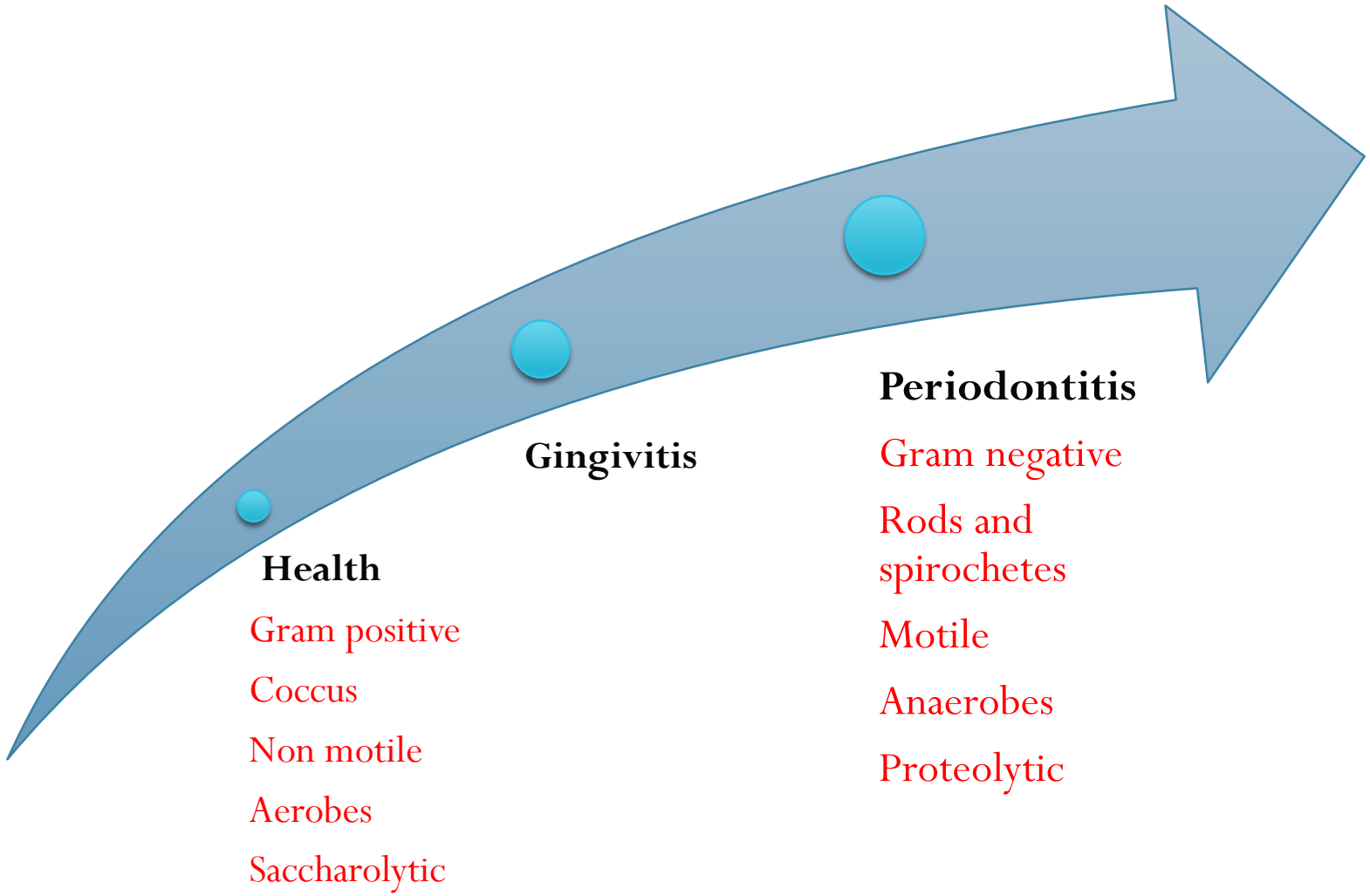
- More effective competition for nutrients and attachment sites,
- The production of inhibitory factors, and
- Creation of unfavorable growth conditions for invading species by the normal micro flora.

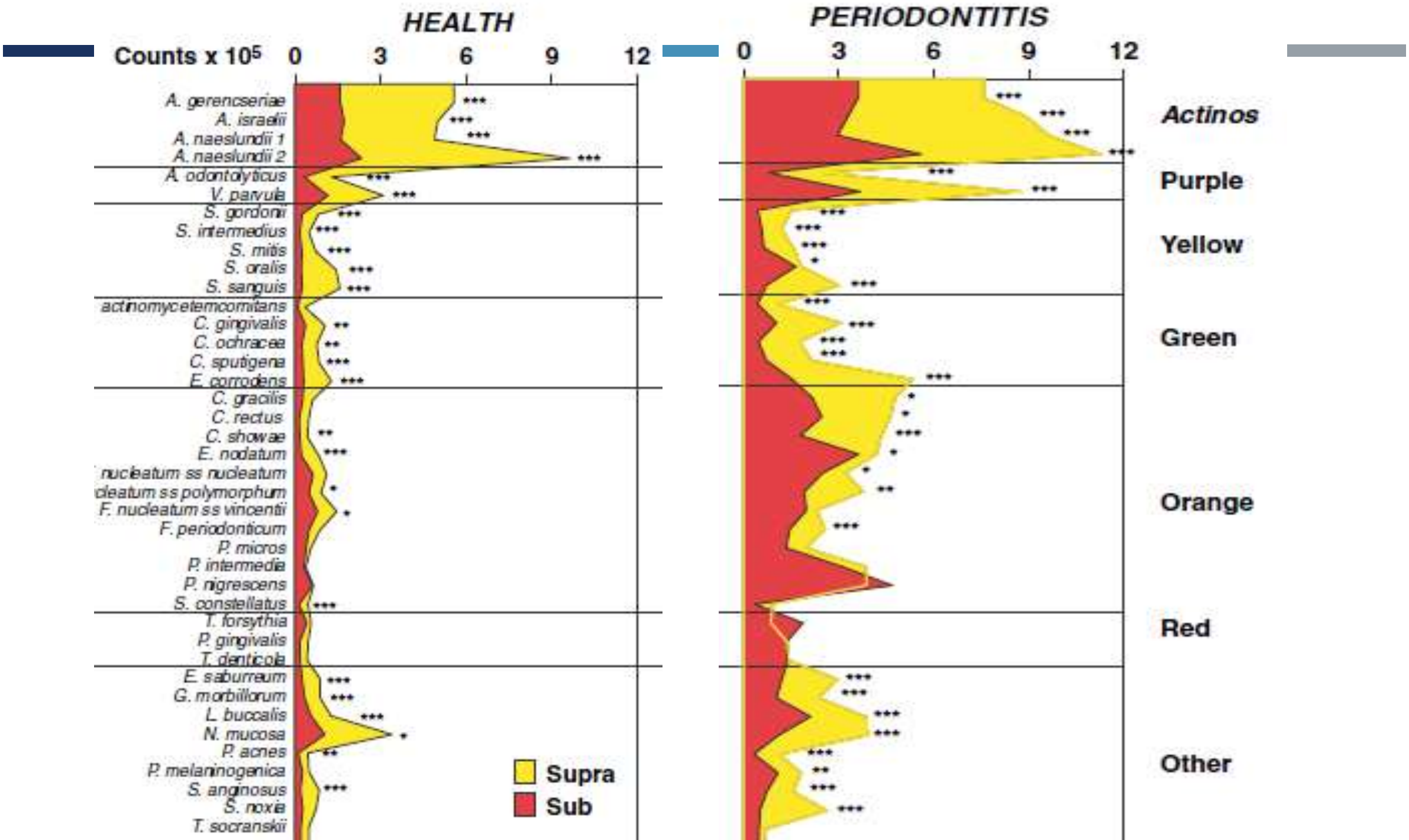
CAUSAL RELATIONSHIP

- Sufficient Cause
- Necessary Cause
- Risk Factor

ROLE OF DENTAL PLAQUE IN DISEASE

- Unlike most classical medical infections, the microflora from sites with periodontal disease is diverse, and no single species is diagnostic or predictive.
- In the absence of effective oral hygiene, plaque can accumulate to levels that are no longer compatible with health, thereby predisposing sites to dental caries or periodontal diseases.
- There is a shift in the balance of the micro flora away from those species that are found at healthy sites .


Health
Gram positive
Coccus
Non motile
Aerobes
Saccharolytic
Gingivitis
Periodontitis
Gram negative
Rods and spirochetes
Motile
Anaerobes
Proteolytic


HEALTH
PERIODONTITIS
Counts x 10^5
0
3
6
9
12
A. gerencseriae
A. israelii
A. naeslundii 1
A. naeslundii 2
A. odontolyticus
V. parvula
S. gordonii
S. intermedius
S. mitis
S. oralis
S. sanguis
actinomycetemcomitans
C. gingivalis
C. ochracea
C. sputigena
E. corrodens
C. gracilis
C. rectus
C. showae
E. nodatum
nucleatum ss nucleatum
cleatum ss polymorphum
F. nucleatum ss vincentii
F. periodonticum
P. micros
P. intermedia
P. nigrescens
S. constellatus
T. forsythia
P. gingivalis
T. denticola
E. saburreum
G. morbillorum
L. buccalis
N. mucosa
P. acnes
P. melaninogenica
S. anginosus
S. noxia
T. socranskii
Supra
Sub
Actinos
Purple
Yellow
Green
Orange
Red
Other

Healthy	Gingivitis	Periodontitis
S. oralis	S. oralis	P. Gingivalis
S. sanguis	S. sanguis	A.actinomycetumcomitans
S. mitis	S. mitis	T. Forsythia
S. mutans	S. Intermedius	T. Denticola
A. naesulundii	C. ochracea	P. Intermedia
A. odontolyticus	C. gingivalis	P. nigrescens
P. micros	Campylobacter gracilis	Campylobacter rectus
E. nodatum	Prevotella loescheii	P. micros
C. ochracea	P. micros	F. Nucleatum
C. gracilis	E. nodatum	Veillonella parvula
	A. naesulundii	
	A.actinomycetumcomitans	

Other periodontal diseases	Associated Microorganism
1. Chronic periodontitis	*P. gingivalis, T. forsythia, P. intermedia, C. rectus, E. corrodens, F. nucleatum* Viruses are also implicated in the etiology of CP
2. Aggressive periodontitis	A. *actinomycetemcomitans* - about 90% Other organisms – *P. gingivalis, E. corrodens, C. rectus, F. nucleatum*
3. Necrotizing periodontal disease	High level of *P. intermedia* and spirochetes
4. Abscesses of periodontium	*F. nucleatum, P. intermedia, P. gingivalis, P. micros*
5. Periimplantitis	Same periodontal pathogens like A.a , P.g also P.aeruginosa, Candida albicans

MICROBES PRESENT IN PLAQUE OTHER THAN BACTERIA

Viruses
Fungi
Protozoa
Archae
Mycoplasma

ROLE OF VIRUSES IN PERIODONTAL DISEASE

In addition to bacteria, many viruses have been implicated in the pathogenesis of periodontal disease.

- Human Immunodefiecncy Virus
- Herpes Simplex Virus
- Human Papilloma Virus
- Ebstein Bar Virus
- Cytomegalo Virus
- Entero Virus species

PROBABLE MECHANISM OF VIRUS ROLE

Virus Activation

Immunosuppression, Infection, Stress, Hormones etc.

↓

Periodontopathic Property

Cytokines, Immunosuppression, Direct Cytotoxicity, Overgrowth of pathogenic Bacteria

↓

Destructive Periodontal Disease

NEWLY IDENTIFIED PERIODONTAL PATHOGENS

- Gram positive with moderate evidence
  - *Eubacterium saphenum*
  - *Mogibacterium timidum*
  - *Peptostre-ptococcus stomatis*
  - *Filifactor alocis*
  - *Enterococcus*

PJP Cheparro et al., 2016

NEWLY IDENTIFIED PERIODONTAL PATHOGENS

- Gram negative with moderate evidence
  - *Porphyromonas endodontalis*
  - *Treponema lecithinolyticum*
  - *Treponema medium*
  - *Tremponema vincentti*
  - *Anaeroglobus geminatus*
  - *Selenomonas sputigena*
  - *Fretibacterium fastidiuosum*

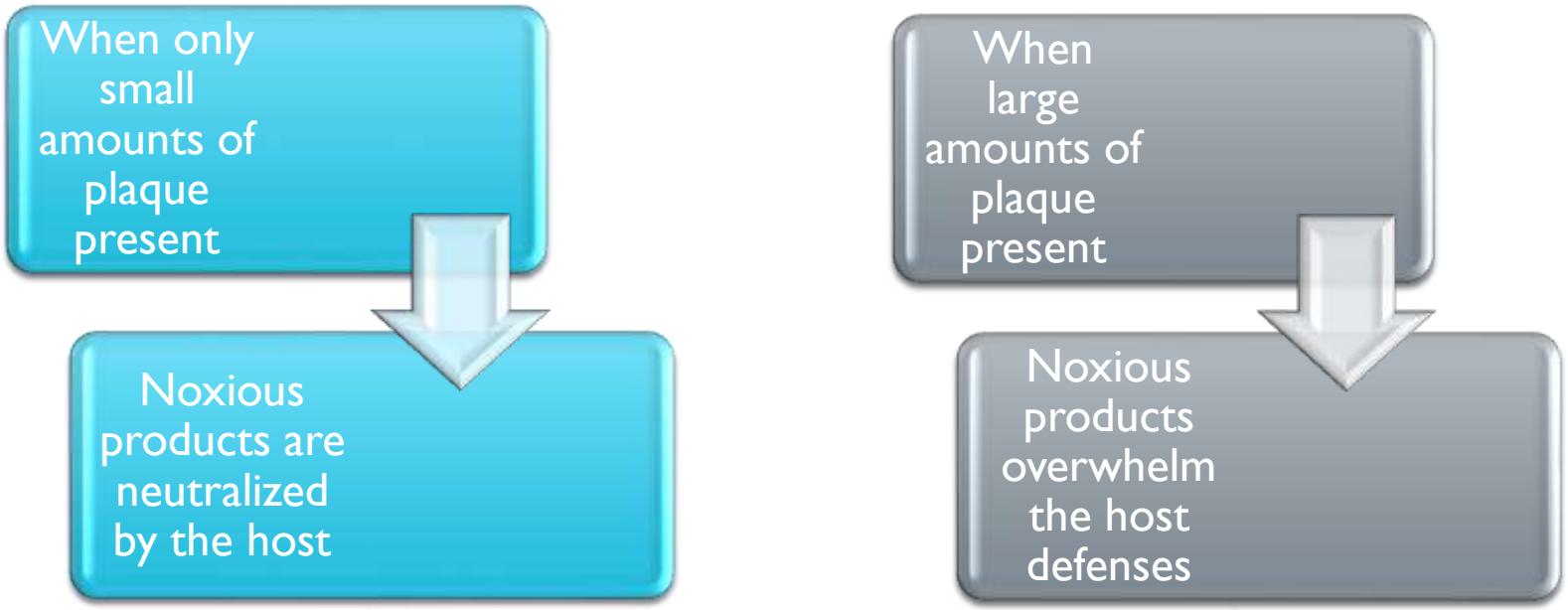
PJP Cheparro et al., 2016

Plaque Hypotheses

NON SPECIFIC PLAQUE HYPOTHESIS

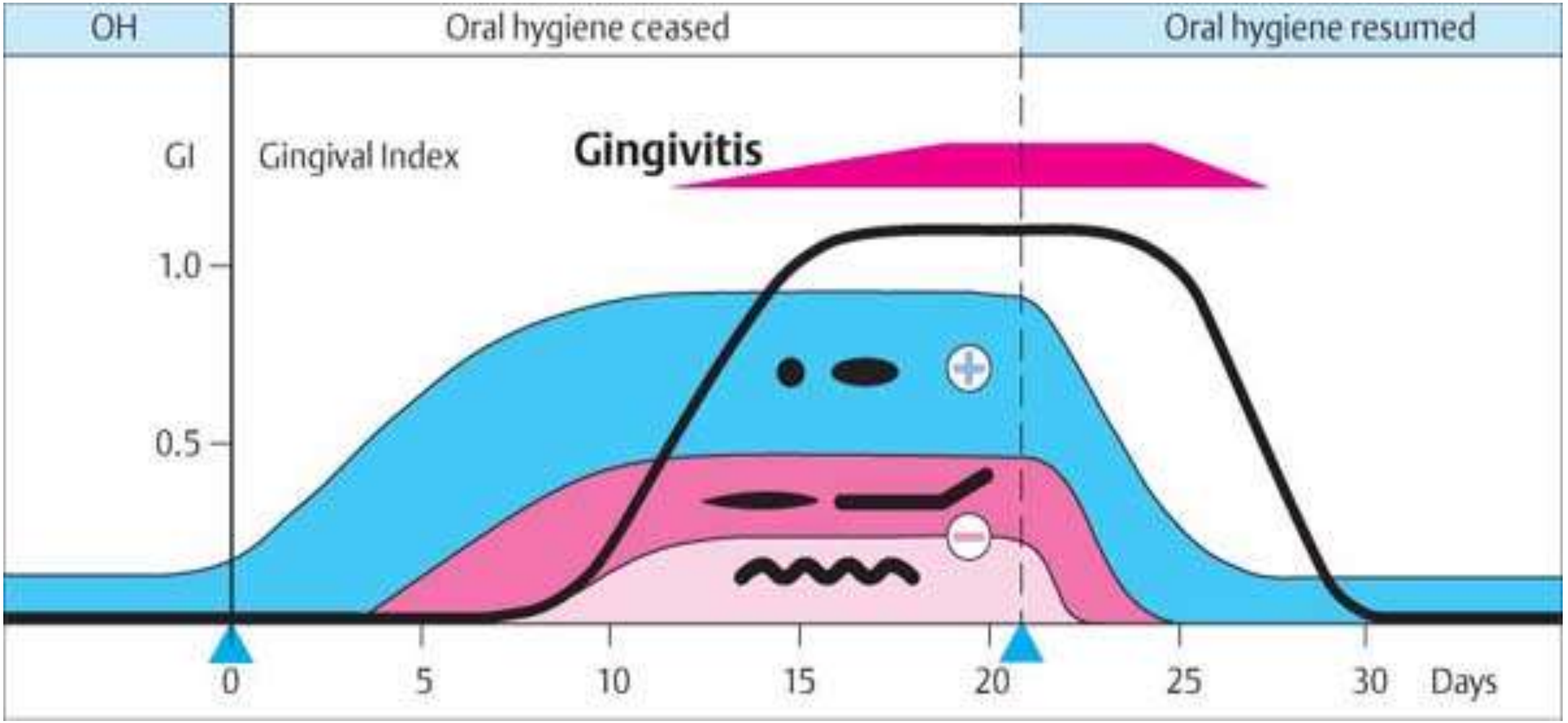
- The nonspecific plaque hypothesis was given in 1976 by W.D. Miller
- According to this hypothesis, any accumulation of micro-organisms at or below gingival margin will produce irritants, leading to inflammation.
- Periodontal disease results from the " *Elaboration of noxious products by the entire plaque flora*.

NON SPECIFIC PLAQUE HYPOTHESIS



Inherent in the nonspecific plaque hypothesis is the concept that control of periodontal disease depends on control of amount of plaque accumulation.

EXPERIMENTAL GINGIVITIS MODEL



SPECIFIC PLAQUE HYPOTHESIS

- Loesche in 1976 proposed that out of the diverse collection of microorganisms that constitute the resident plaque microflora, only a very *limited number* are actively involved in causing disease.

- "Only *certain plaque is pathogenic* and its pathogenicity depends on the presence of or an increase in, *specific microorganisms*.

Inherent in this theory is the belief that periodontal disease results from specific bacteria found in dental plaque that is different in composition from dental plaque found in healthy sites.

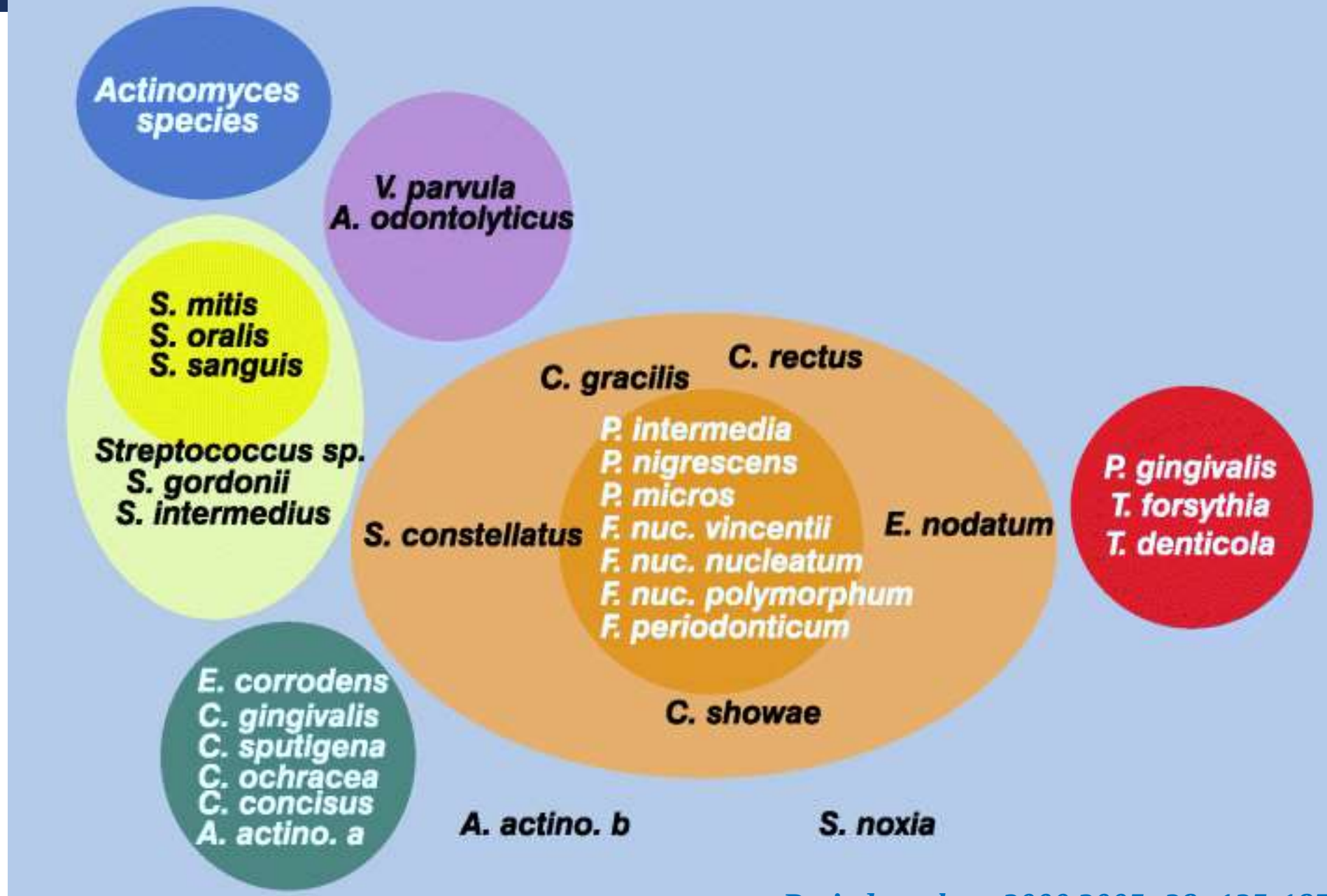
UNIFIED THEORY

- In the modern version of the specific theory, *Socransky* in 1979 abandoned the idea of a single periodontal pathogen and stated that *periodontal disease can be initiated by a number of specific microorganism.*

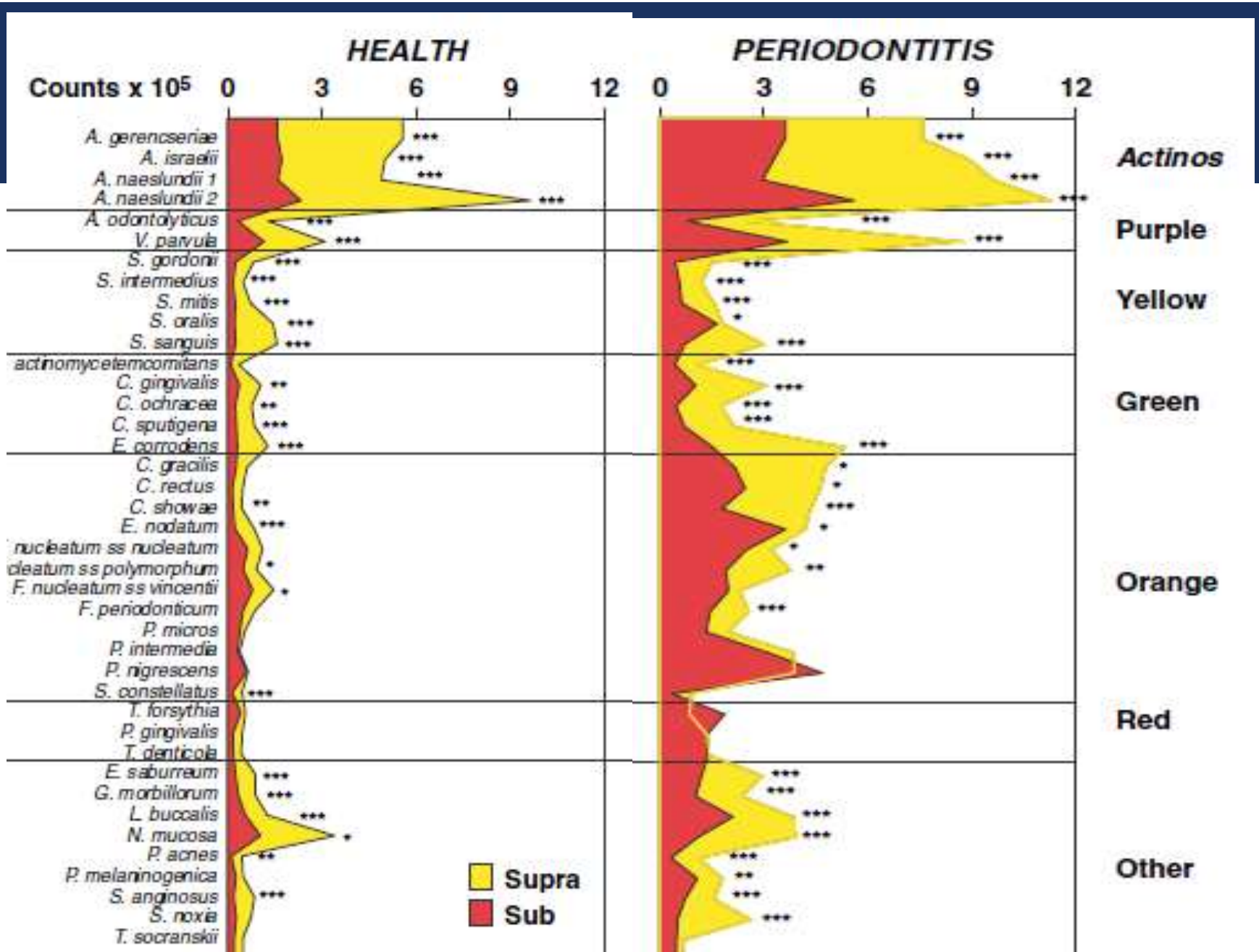
- He stated that 6-12 bacterial species may be responsible for the majority of cases of destructive periodontitis and additional species may be responsible for a small number of other cases.

EVIDENCE FOR SPECIFIC PLAQUE HYPOTHESIS

Periodontal diseases	Specific Microorganism
1. Necrotizing periodontal disease	High level of *P. intermedia* and spirochetes
2. Aggressive periodontitis	A. *actinomycetemcomitans* - about 90% Other organisms – *P. gingivalis, E. corrodens, C. rectus, F. nucleatum*
3. Chronic periodontitis	*P. gingivalis, T. forsythia, P. intermedia, C. rectus, E. corrodens, F. nucleatum* Viruses are also implicated in the etiology of CP



Periodontology 2000 2005; 38: 135-187


HEALTH
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9
12
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A. israelii
A. naeslundii 1
A. naeslundii 2
A. odontolyticus
V. parvula
S. gordonii
S. intermedius
S. mitis
S. oralis
S. sanguis
actinomycetemcomitans
C. gingivalis
C. ochracea
C. sputigena
E. corrodens
C. gracilis
C. rectus
C. showae
E. nodatum
nucleatum ss nucleatum
cleatum ss polymorphum
F. nucleatum ss vincentii
F. periodonticum
P. micros
P. intermedia
P. nigrescens
S. constellatus
T. forsythia
P. gingivalis
T. denticola
E. saburreum
G. morbillorum
L. buccalis
N. mucosa
P. acnes
P. melaninogenica
S. anginosus
S. noxia
T. socranskii
Supra
Sub
Actinos
Purple
Yellow
Green
Orange
Red
Other

DENTAL DISEASES AND ECOLOGICAL PERSPECTIVE

- Mouth is a unique micro habitat
- It has multiple biological niches with multi-species
- “Selection” of “pathogenic” bacteria among microbial community depends on ***environmental changes***

ECOLOGICAL PLAQUE HYPOTHESIS

- Was proposed by *Marsh 1991* in an attempt to unify various clinical and laboratory observations.
- He proposed that a change in a *key environmental factor* can trigger a shift in the balance of the resident plaque microflora.
- Under the conditions that prevail in health, these organisms would be only weakly competitive and may also be suppressed by inter microbial antagonism so that they would compromise only a small percentage of the plaque microflora and would not be significant clinically.

ECOLOGICAL PLAQUE HYPOTHESIS

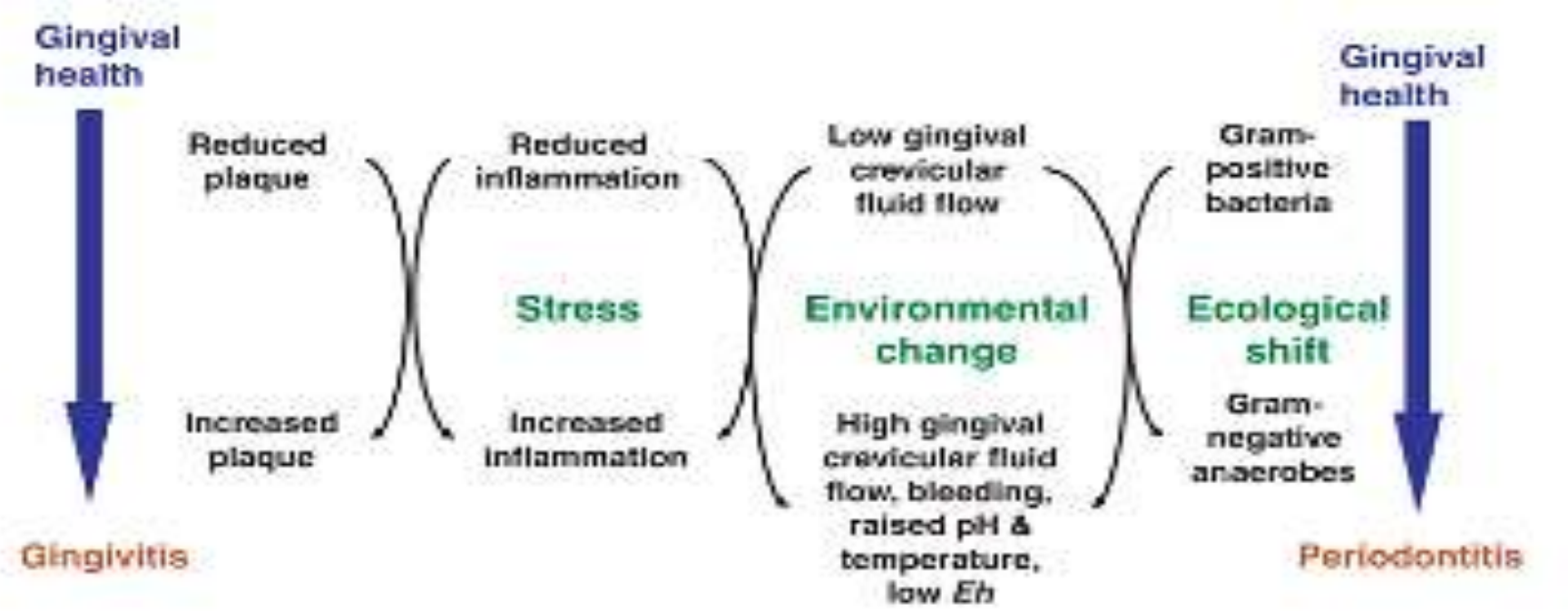
- Microbial specificity in disease would be due to the fact that only certain species are competitive under the new environmental conditions.

- It is a basic tenet of microbial ecology that a major change to an ecosystem produces a corresponding disturbance to the stability of the resident microbial community

(Brock 1966; Alexander 1971, Fletcher et al 1987).

ECOLOGICAL PLAQUE HYPOTHESIS

Marsh PD: Oral ecology and its impact on oral microbial diversity; in Kuramitsu HK, Ellen RP (eds): Oral Bacterial Ecology: The Molecular Basis. Wymondham, Horizon Scientific Press, 2000a, pp 11–6



Disease is due to a change in local environmental conditions, which disrupts the natural balance between plaque and the host, leading to the enrichment of organisms that can cause disease

"Pathogen along with local environment is responsible for disease"

KEYSTONE PATHOGEN HYPOTHESIS

- The concept of keystone species is derived from basic ecological studies. Certain species have an effect on their environment that is disproportional relative to their overall abundance.

(Paine,1969;Poweretal.,1996;Darveauetal.,2012).

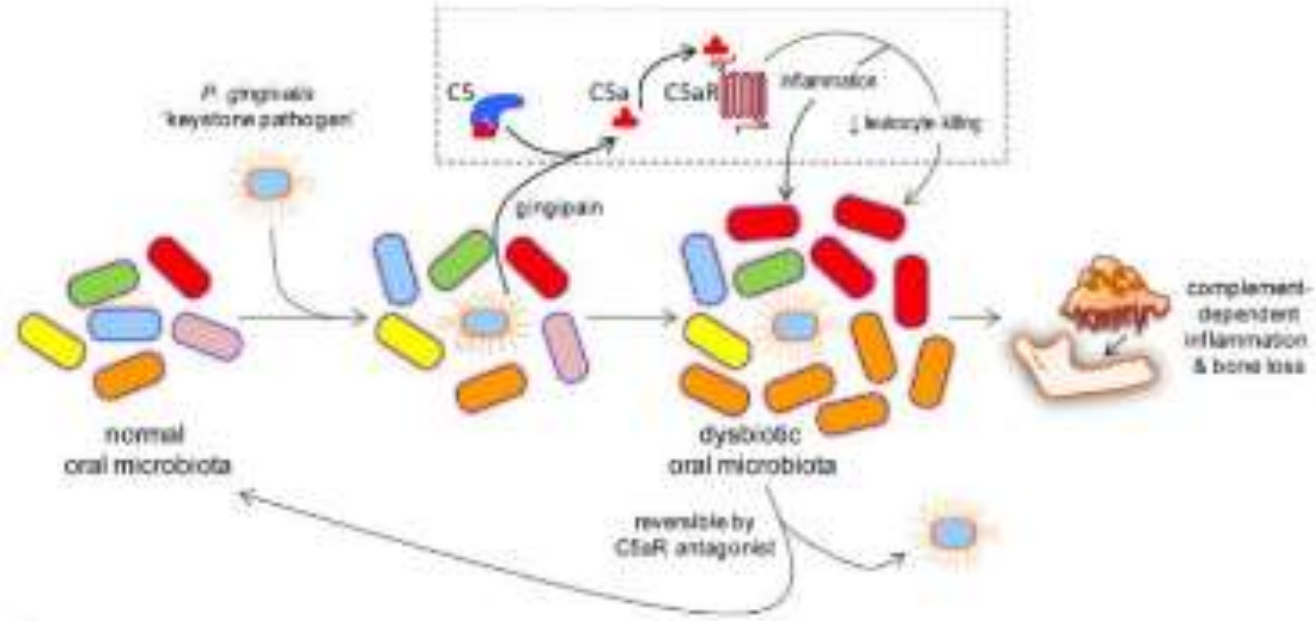
- George Hajishengallis and colleagues (2012) applied this concept to (oral) microbiology by proposing “The Keystone-Pathogen Hypothesis”

(Hajishengallisetal.,2012).

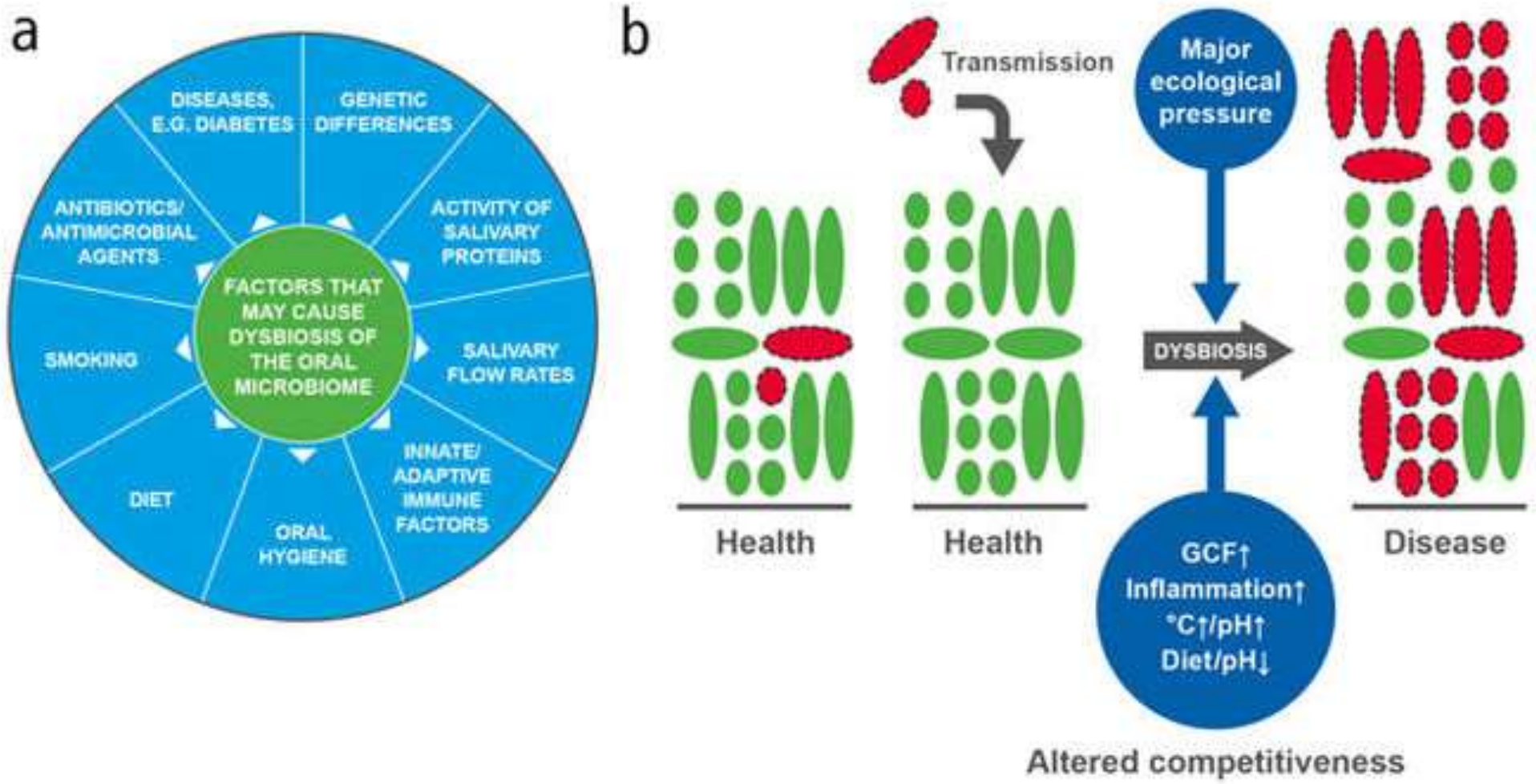
- The KPH indicates that certain low abundance microbial pathogens can cause inflammatory disease by increasing the quantity of the normal microbiota and by changing its composition

(Hajishengallisetal.,2012).

KEYSTONE PATHOGEN HYPOTHESIS

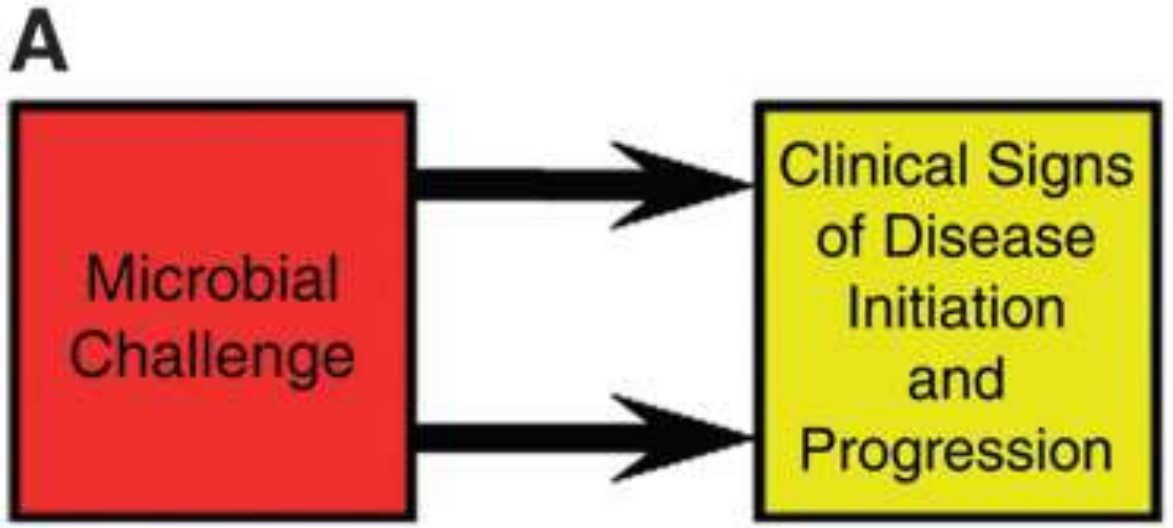


KEYSTONE HYPOTHESIS



PERIODONTAL DISEASE MODELS

EARLY BACTERIAL MODEL OF PERIODONTAL DISEASE

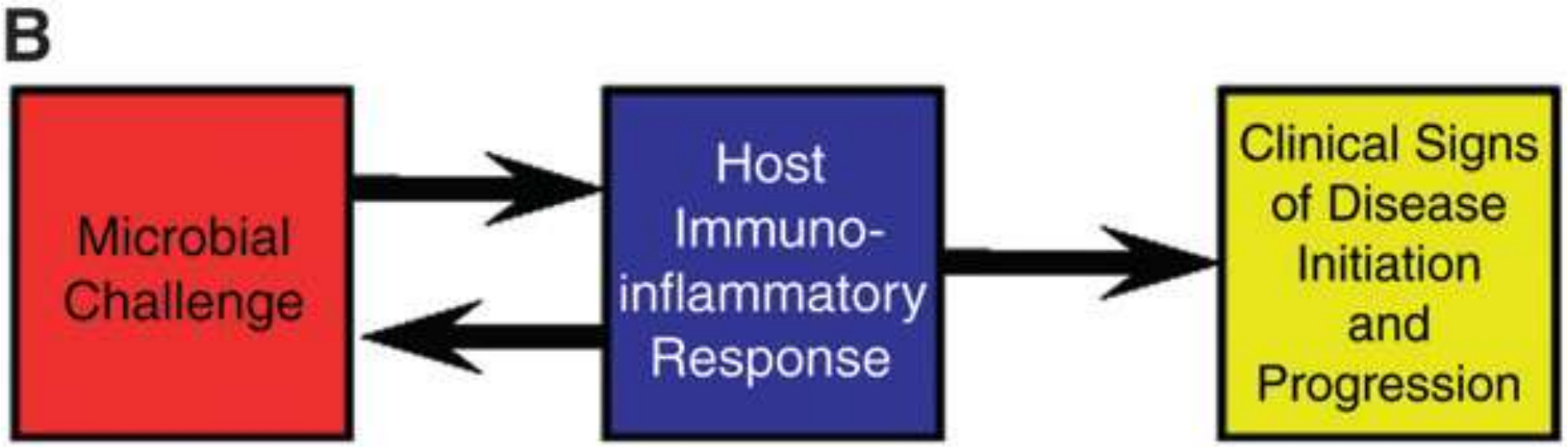


IMPLICATED BACTERIAL PLAQUE DEPOSITS AS THE PRIMARY DIRECT FACTOR IN THE DEVELOPMENT OF PERIODONTITIS

Direct effects of damage by bacteria

- Evasion of host defense
- Degradation of Immunoglobulin
- Modulation of cytokine function
- Degradation of fibrin
- Altered lymphocyte function
- Damage to crevicular epithelium
- Production of volatile Sulphur compounds
- Degradation of periodontal tissue by enzymes

LINEAR MODEL OF PERIODONTAL DISEASE



MODEL DEPICTING HOST MICROBIAL INTERACTION

Indirect mechanism of tissue damage : **Immunity**

Bacterial antigens penetrate the crevicular epithelium

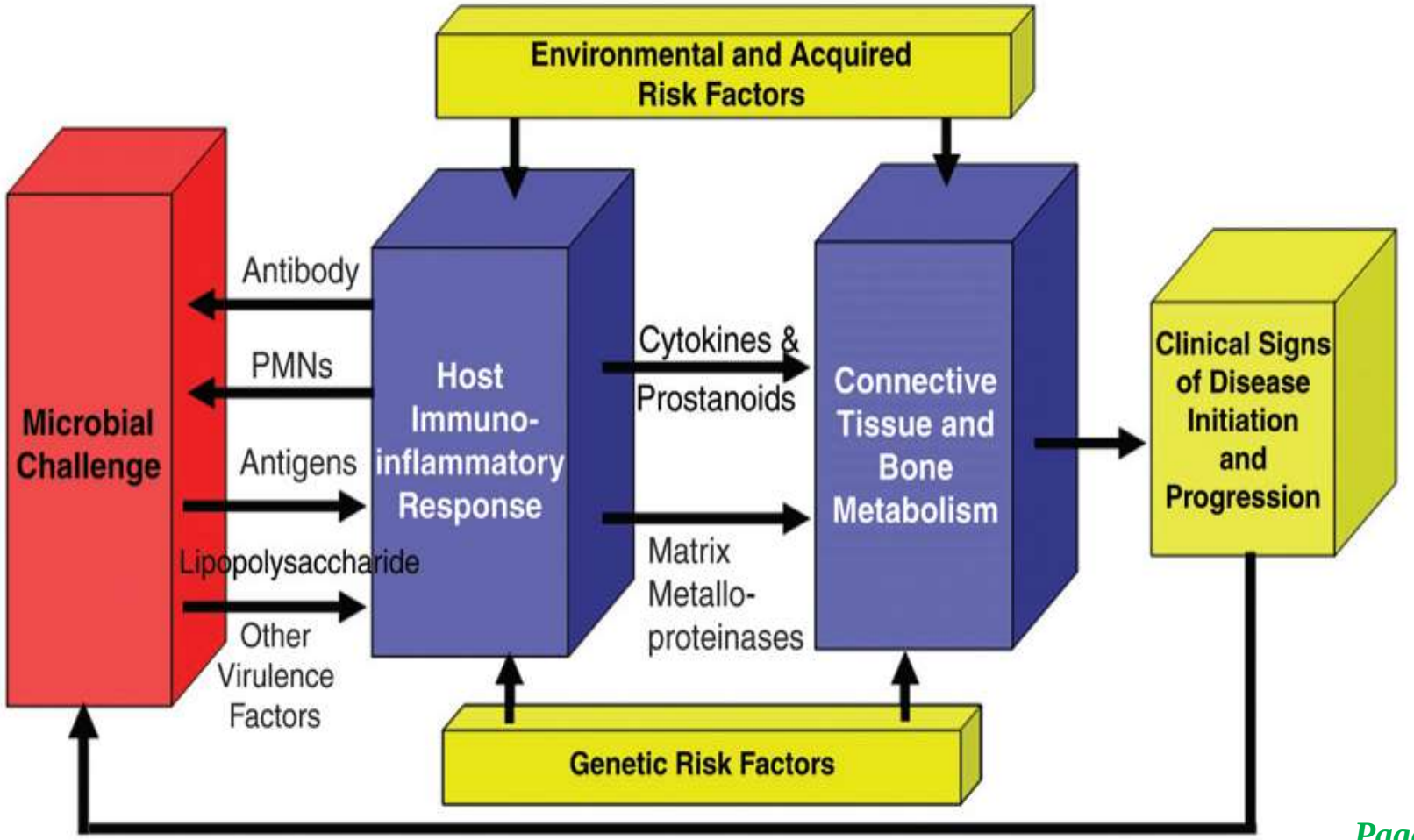
Enter tissues and stimulate humoral immune response

Accumulation of plasma cells and production of immunoglobulin

Activation of complement cascade release of prostaglandins

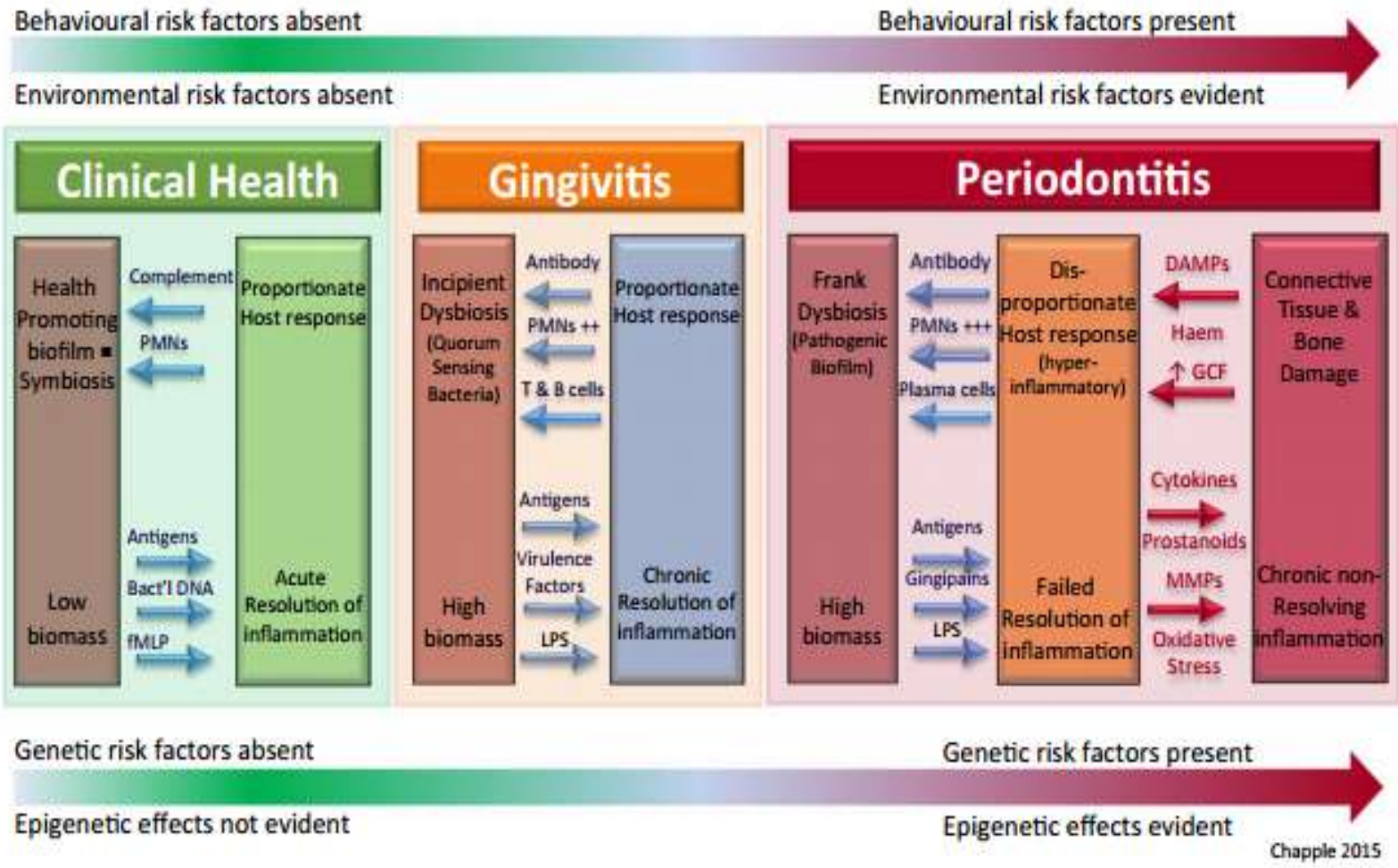
The accumulated inflammatory cells can release tissue destructive enzymes

NON LINEAR MODEL OF PERIODONTAL DISEASE



Page RC & Kornman KS - 1997

POLYMICROBIAL SYNERGY AND DYSBIOSIS MODEL



CLINICAL IMPACT OF BIOFILM

- Oral disease management should move towards approaches that have proved successful in many areas of medicine.
- The clinical approach should be more holistic and a patient should be considered a holobiont.
- Attempt should be made to monitor and manipulate the composition and metabolism of the oral microbiome in order to maintain the beneficial activity we derive from their presence, while minimizing the impact of any environmental and lifestyle factors that might lead to dysbiosis in the future

SUMMING UP……

- Dental researchers have attempted to understand the microbial nature of oral diseases over the past 120 years.
- Recently dental researchers have begun to view plaque as a biofilm and found that dental biofilm is a primary etiologic factor for the most frequently occurring oral diseases, dental caries and periodontal diseases
- The view of plaque and its constituent microorganisms has shifted over the years from non-specific to specific, ecological and disbiotic plaque hypothesis.

SUMMING UP……

- The nature of a biofilm helps explain why periodontal diseases have been so difficult to prevent and treat.

- In order to adjust a complex microbial ecosystem to one that is compatible to health, it is essential to define the range of species that colonize the area, recognize their relationship with each other and the host, and develop effective strategies to guide these ecosystems to those compatible with long-term oral health.

- An improved understanding of biofilm will lead to new strategies for management of these widespread diseases

THANK YOU!

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