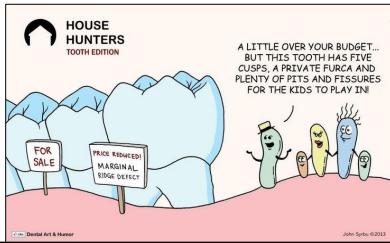


 SCHOOL OF DENTISTRY
Oregon Health & Science University

Research Correlate

Lecture 1 – Microbiology of caries

OPTH725 – Caries
Dr. Justin Merritt
MRB 424
merrittj@ohsu.edu



1

Goals for the 2 lectures

- Understand the basic biology of dental plaque formation
- Connect the ecology within dental plaque to oral health status
- Understand how certain bacterial species are able to prevent or promote caries
- Based upon the above, connect why current caries research themes are of interest
- Understand the modern techniques employed
- Ultimately, understand how caries research could be translated therapeutically

2

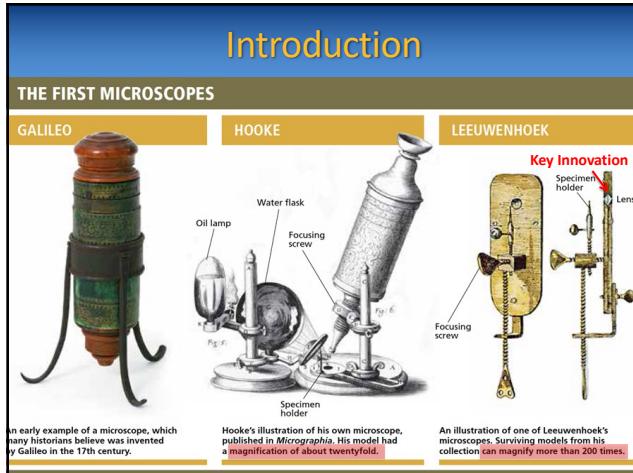


3

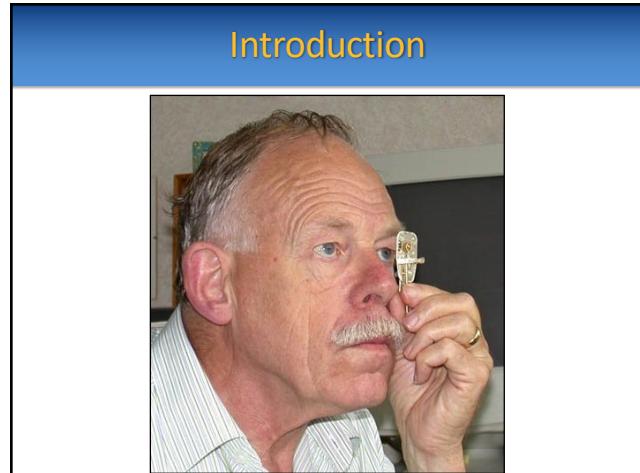
Introduction

- Father of microbiology - Anton van Leeuwenhoek
- 1st person to view and describe bacteria in a letter to the Royal Society of London in 1683
- Leeuwenhoek's lens-making technique was his key innovation – his microscope offered much better resolution than others at the time
- **What were the first bacteria ever viewed?**
- His description of plaque: "a little white matter, which is as thick as if 'twere batter."

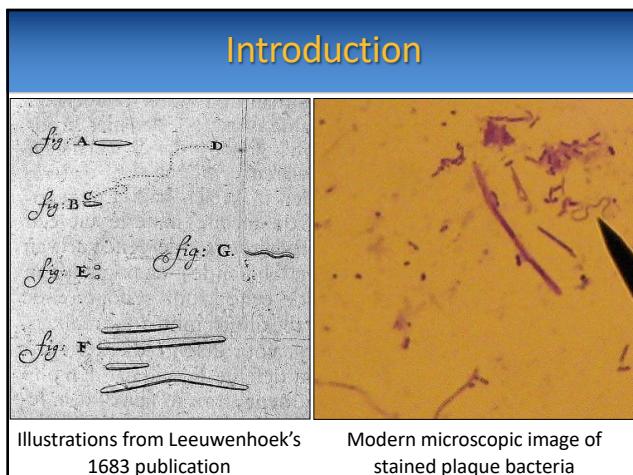
4



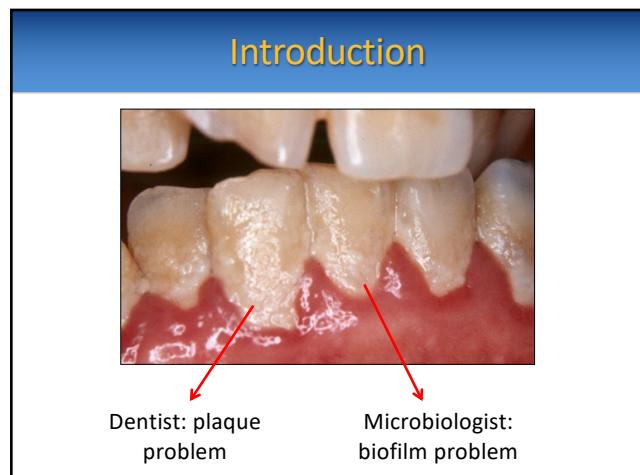
5



6



7



8

Biofilm factoids

- Community of surface attached cells encased in an EPS matrix composed of polysaccharides, proteins, lipids, and DNA (EPS constitutes 50-95% of biofilm dry weight)
 - For bacteria, biofilms are the **norm** not the exception
 - Cells growing in a biofilm exhibit major differences in behavior as compared to their free-living state (planktonic)
 - Distinct genetic program (planktonic vs. biofilm)
 - Bacteria constitute 15 – 20% of biofilm volume (Avg.)
 - Nonrandom distribution of cells in “microcolonies”
 - **Clinical Relevance:** NIH estimate: 80% of *all* human bacterial infections are biofilm related
 - **Clinical Relevance:** Biofilms are extremely resistant to all types of environmental insults (including antibiotics)

9

Dental Plaque

- Dental plaque is a multispecies biofilm formed by the oral flora on the tooth surface
 - Plaque population is very diverse (hundreds of species possible) and mostly bacterial species
 - Subgingival (below the gumline) and supragingival plaque (above the gumline) populations are distinct
 - Cell density is extremely high: $10^{10} - 10^{12}$ CFU/mL
 - Why has nature allowed our mouths to host so much bacterial growth? Is there a benefit?

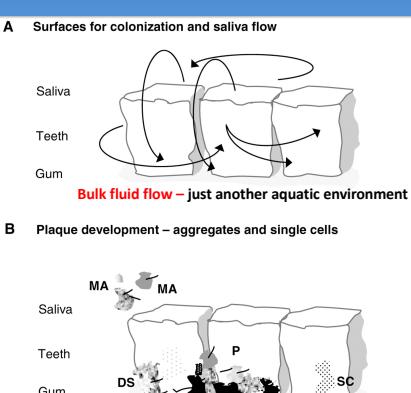
10

Plaque Development

Saliva contains proteins that aggregate bacteria

Why do this?

Many bacterial aggregates floating around the oral cavity



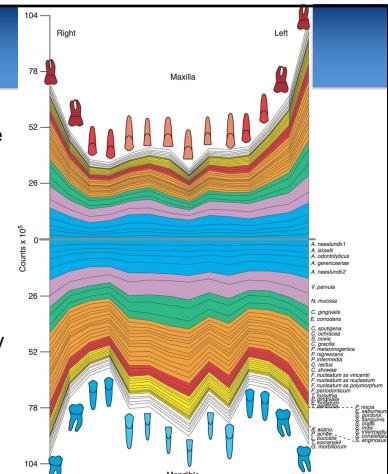
11

Site-specific Ecology

Supragingival plaque samples taken from the mesiobuccal surface from 187 subjects

40 bacterial species
counted from each
sample

Data illustrate variability
of both plaque
accumulation and
species composition
among teeth



12

Biofilm and Plaque summary

- Biofilm bacteria have distinct characteristics from free living bacteria
- Plaque is a biofilm composed of oral flora on the teeth
- The plaque population differs depending on location and health status

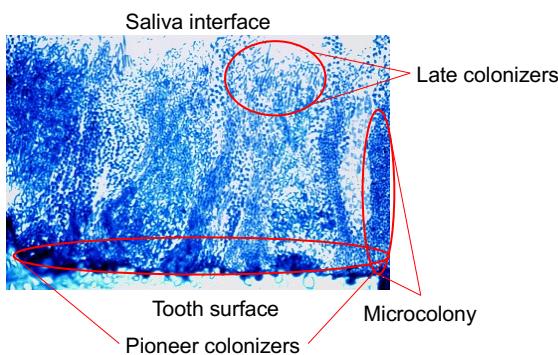
13

Overview of Plaque Development

- Dental plaque is formed in 4 discreet steps:
- 1. Formation of acquired pellicle
- 2. Adherence of pioneer colonizers to the pellicle
- 3. Coaggregation/coadhesion of bridging organisms to pioneer colonizers
- 4. Coaggregation/coadhesion of the late colonizers to bridging species
- This sequence of events is also a major driver of plaque ecology

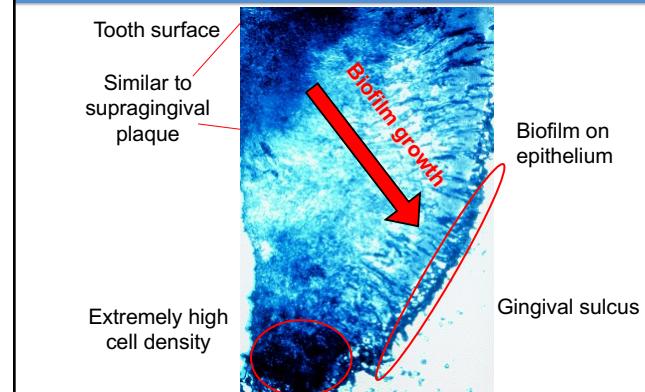
14

Stained 3-day supragingival plaque

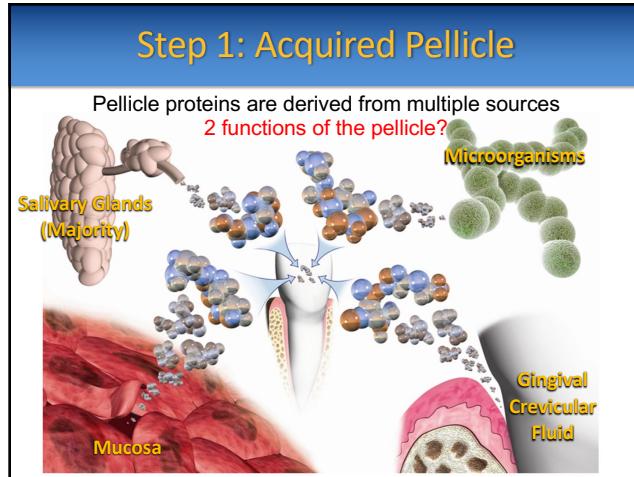


15

Stained 3-day subgingival plaque



16



17

Step 2: Pellicle Adhesins of Oral Flora

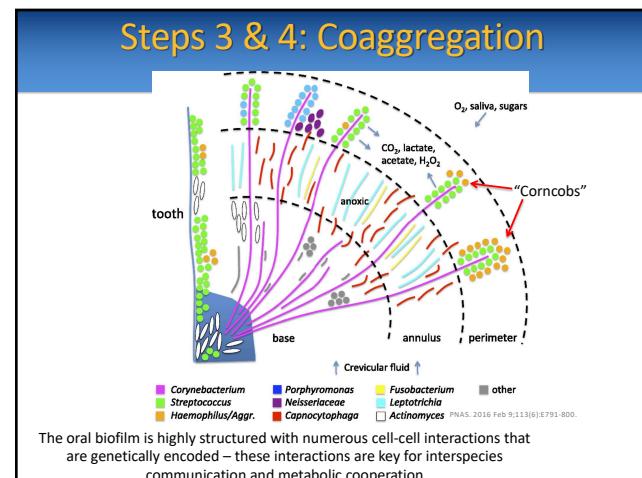
Adhesin	Approx. size (kDa)	Species	Substrate
Antigen I/II family [Ag I/II, AgB, P1 (SpaP), Pac, Sr, SpaA, Pag, SspA, SspB, ScaA]	160–175	<i>S. mutans</i> , <i>S. sobrinus</i> , <i>S. gordonii</i> , <i>S. oralis</i> , <i>S. intermedius</i>	parotid salivary agglutinin (SAG), salivary glycoproteins, proline-rich proteins, collagen
Lal family [FimA, SsaB]	35	<i>S. parasanguis</i> , <i>S. sanguis</i>	salivary components in pellicle
Fap1	200	<i>S. parasanguis</i>	salivary components in pellicle
Amylase-binding proteins	82, 65, 20, 15, 12	<i>S. gordonii</i> , <i>S. mitis</i> , <i>S. crista</i>	α amylase
Antigen complex	80, 62, 52	<i>S. gordonii</i>	73-kDa submandibular salivary protein
Surface lectins	96, 70, 65	<i>S. oralis</i> , <i>S. mitis</i>	salivary glycoprotein presenting N-acetylneurameric acid
Type 1 fimbriae-associated protein		<i>A. naeslundii</i>	proline-rich proteins, statherin

Notice these are all *Streptococcus* and *Actinomyces* species.
Why is this?

18

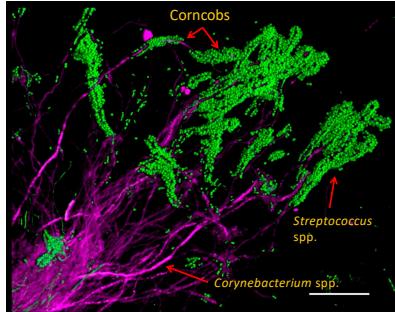
- ### Steps 3 & 4: Coaggregation
- Coaggregation refers to the ability of certain plaque species to specifically associate with each other (coadhesion)
 - Associations usually involve adhesins and receptors
 - Largely responsible for the microbial diversity seen in plaque
 - What are some reasons that might explain why bacteria have this ability?

19



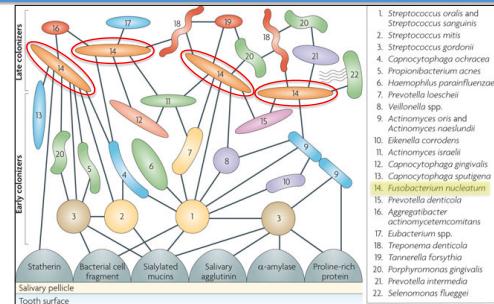
20

Steps 3 & 4: Coaggregation in Clinical Samples



FISH stained fluorescent image of human dental plaque
We will discuss FISH staining later...

Steps 3 & 4: Coaggregation with Bridge Species



Fusobacterium species are by far the most promiscuous at binding to other organisms. They are considered the glue that holds the mature plaque together ("bridge species").

21

22

Plaque Summary

- Plaque development occurs stepwise
- The acquired pellicle provides the initial site of attachment to begin plaque development
- Specific species of bacteria are known to associate with each other (coaggregate)
- Due to successive colonization and coaggregation/coadhesion, distribution of species throughout the plaque is *not* random

Perspective

- Why is plaque structured as it is?
- Plaque functions as a microbial ecosystem
- Ecological plaque hypothesis:
- Perturbations of this ecosystem are the cause of dental caries (and periodontitis too)
- Dysbiosis – alteration of the flora ecology; often results in pathology
- Dysbiosis is the root of many (if not most) diseases that occur at mucosal sites

23

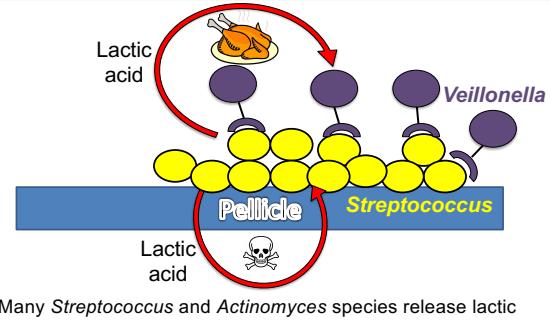
24

Plaque Ecology

- Oral cavity functions as an aquatic ecosystem
- As with many ecosystems, high species diversity ensures that all ecological niches are filled
- “**Niche**”: a particular growth environment in which an organism can proliferate
- Many species have a specialized ability to exploit specific niches
- The growth of one species creates niches for another (new energy sources or a more favorable local environment)
- If a species is removed from the system, it can impact many others

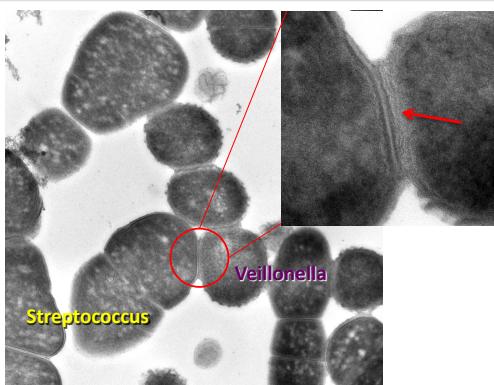
25

Plaque as a Community



26

Plaque as a Community



27

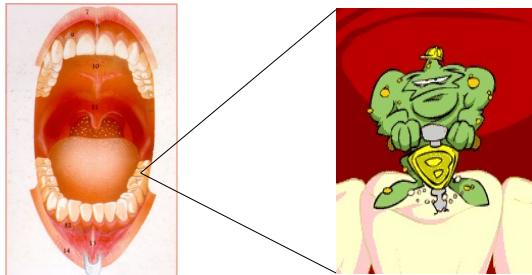
Plaque as a Community

- Each species has evolved as specialized ability to inhabit a specific niche
- Each species contributes to the proper functioning of the community
- This specialization of function in plaque is quite analogous to city life
- If essential functions are lost in the city (medicine, sanitation, shipping, etc....), the system progressively breaks down
- Caries is the byproduct of community disintegration in dental plaque

28

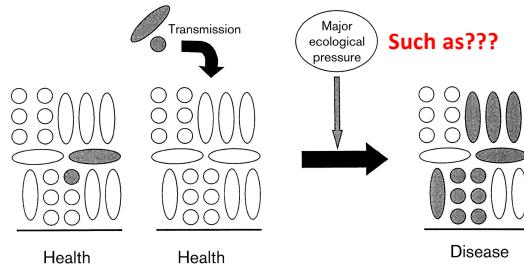
The Big Question...

If the plaque community is such a well tuned machine, what might cause the system to fall apart and initiate disease?



29

Caries Initiation



Disease results from a major change in the population composition

30

Plaque Ecology – Commensal vs. Pathobiont



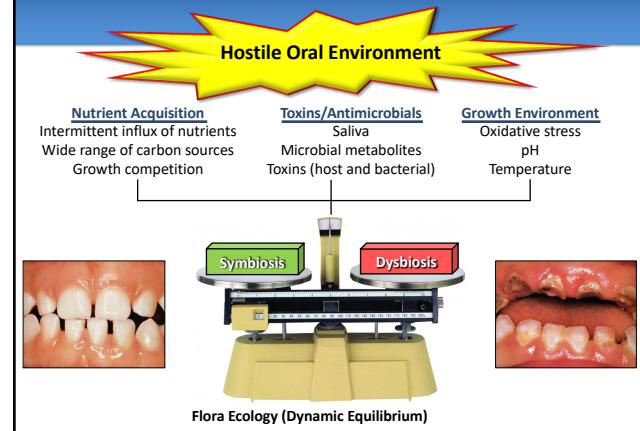
Not all members of the microbiome are advantageous in large numbers.
Microbiome composition is not static – Influenced by the oral environment.

Commensal: health-promoting in large numbers; protect against disease
Examples: *Streptococcus mitis*, *Streptococcus sanguinis*, *Streptococcus salivarius*

Pathobiont: low abundance species in health; high abundance in disease
Examples: *Streptococcus mutans*, *Porphyromonas gingivalis*

31

Plaque Ecology – Commensal vs. Pathobiont



32

Commensal Flora – H₂O₂ Production

PIONEER COLONIZERS (Commensals)

Streptococcus gordonii, *S. sanguinis* (SK36), *S. mitis* → H₂O₂ (hydrogen peroxide) → **CARIOGENIC (Pathobionts)**

S. mutans, Other cariogenic species

The diagram shows two panels. The left panel shows three circles representing bacteria: *S. mutans* (grey), SK36 (white), and *S. mitis* (grey). A red circle around the SK36 circle is labeled "Growth inhibition". The right panel shows the same setup, but the SK36 circle is labeled "Peroxide deficient mutant (No inhibition)" with a red arrow pointing to it.

Many pioneer colonizers (especially the Mitis group streptococci) produce H₂O₂ – inhibits *S. mutans* and other cariogenic species, but not other pioneer colonizers.

33

Commensal Flora – H₂O₂ Production

Spot assays showing H₂O₂ production by *S. mutans* (Sm) and *S. oligofermentans* (So). Panel A shows growth of both strains in PBS. Panel C shows growth of Sm but not So in the presence of peroxidase, indicating H₂O₂ production by Sm.

S. oligofermentans is a Mitis group pioneer colonizer originally isolated from caries-resistant patients. It has 2 major H₂O₂ producing enzymes, pyruvate oxidase (Pox) and lactate oxidase (Lox).

The *S. oligofermentans* Lox enzyme uses the lactic acid produced by *S. mutans* as a substrate to produce H₂O₂ and kill *S. mutans*.

34

Commensal Flora – NH₃ Production

Diagram illustrating the shift in oral pH from neutral (Health) to low pH (Disease) due to caries. Oral environmental stresses lead to acidification. Oral alkali generation by commensals (represented by open circles) helps neutralize acid, while acidogenic bacteria (represented by filled circles) contribute to acidification. This balance is disrupted during disease, leading to caries.

Int J Oral Sci. 2012 Sep;4(3):135-40.

Production of alkali (NH₃) neutralizes acid: removes the growth advantage of cariogenic species and prevents tooth demineralization

35

Commensal Flora – NH₃ Production

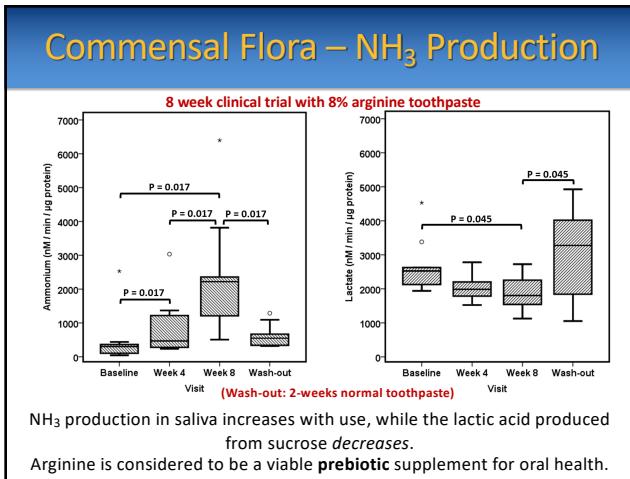
Arginine Deiminase System (ADS) of commensal streptococci

The diagram shows the ADS pathway. Arginine is converted to Citrulline by Arginine deiminase, releasing NH₃. Citrulline is then converted to Agmatine by Arginine decarboxylase, releasing CO₂. Agmatine is converted to Carbamoyl putrescine by Agmatine deiminase, also releasing NH₃. Carbamoyl putrescine is converted to Putrescine by Putrescine carbamoyl transferase, using Ornithine carbamoyl transferase and Carbamate kinase. Putrescine is then converted to Putrescine carbamoyl transferase, using Ornithine carbamoyl transferase and Carbamate kinase. Putrescine is then converted to Putrescine carbamoyl transferase, using Ornithine carbamoyl transferase and Carbamate kinase.

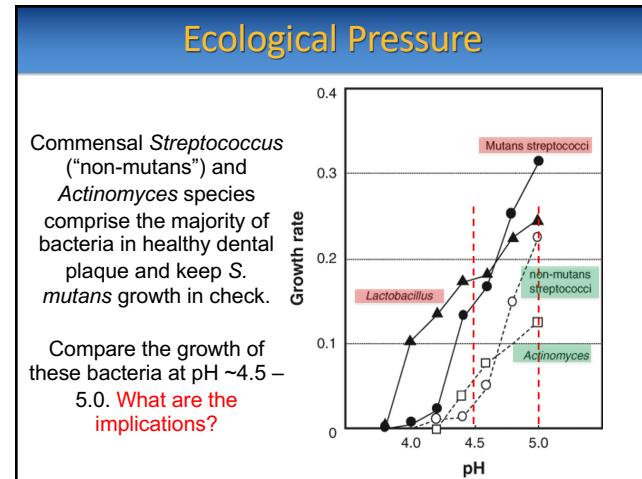
The commensal streptococci are not as resistant to low pH as *S. mutans*, but are uniquely adept at producing NH₃.

S. mutans is missing most of these NH₃-generating pathways.

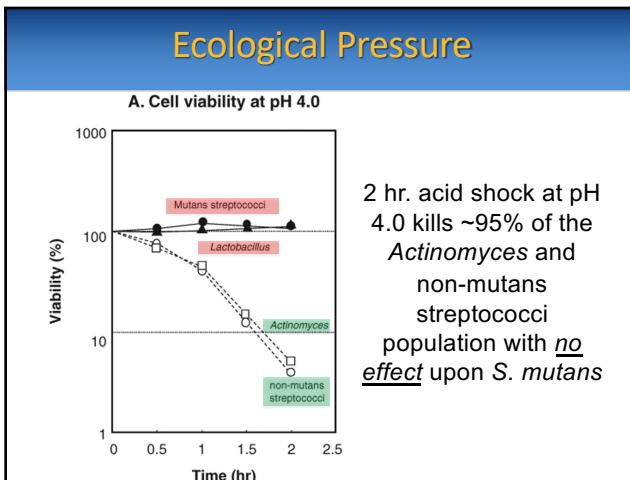
36



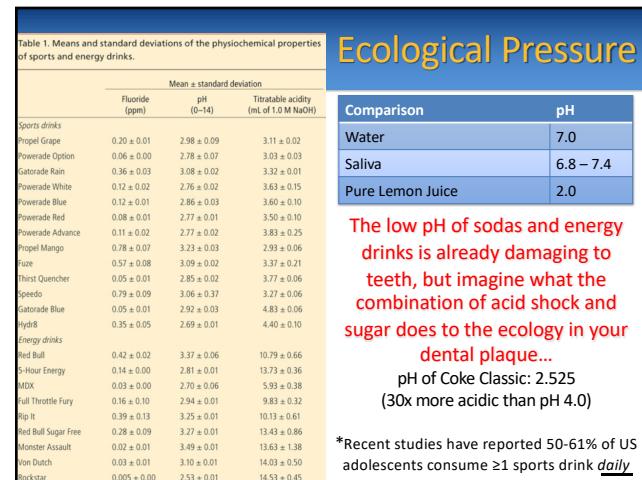
37



38



39



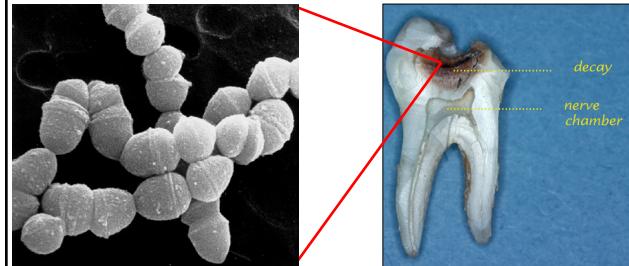
40

Cariogenesis

- Current data suggests that there is no specific caries pathogen
- Multiple species are routinely detected in carious lesions and therefore are *correlated* with disease occurrence
- Streptococcus mutans*** is one of the most frequently detected species in carious lesions and among the most abundant
- S. mutans* is the most studied cariogenic species
- Caries has been observed in sites where *S. mutans* was not detectable

41

Cariogenic Species



What properties allow *S. mutans* to be such a successful cariogenic species?

42

Acid Production (Acidogenic)

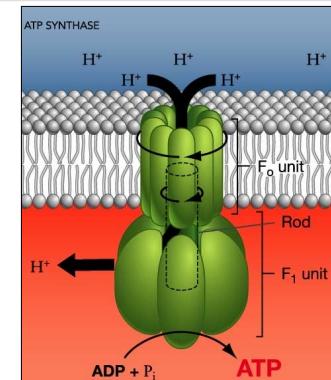
Table. Acidogenicity of Representative Caries-associated Bacteria

Bacteria	Final pH	Reference
Non-mutans streptococci	4.2-5.2 °	1
<i>Actinomyces</i>	4.3-5.7 °	2
Mutans streptococci	4.0-4.4 °	1
<i>Lactobacillus</i>	3.6-4.0 °	1
<i>Bifidobacterium</i>	3.9-4.0 °	3

pH of liquid culture after growth in glucose containing medium
S. mutans is very acidogenic, but others are more so

43

Acid Tolerance (Aciduric)



Many organisms in all kingdoms of life use the F1FO ATPase to generate ATP



S. mutans is unusually proficient at performing the reverse reaction.
 What happens in reverse?

44

Glucosyltransferase

Sucrose

Dextrans/Glucans

Gtf enzyme activity

Glucosyltransferase can split sucrose into glucose and fructose and then polymerize the glucose residues into an exopolysaccharide referred to as dextrans/glucans

45

Glucosyltransferase

Dextrans – mostly $\alpha(1-6)$
Glucans – mostly $\alpha(1-3)$
Linkages influence the behavior
Of the polymer

46

Glucosyltransferase

Gene	Glucan	Size (aa)	MW (KD)
<i>gtfB</i> Glucans	87% $\alpha(1-3)$ (water insoluble) 13% $\alpha(1-6)$ (water soluble)	1475	150
<i>gtfC</i> Glucans	85% $\alpha(1-3)$ 15% $\alpha(1-6)$	1375	140
<i>gtfD</i> Dextrans	30% $\alpha(1-3)$ 70% $\alpha(1-6)$	1430	155

The GTFs are secreted enzymes that primarily create insoluble exopolysaccharide (glucan).
Why do you suppose they do this?

47

Glucosyltransferase

Glucans are extremely sticky and adhere tenaciously to the tooth surface. *S. mutans* has the ability to bind to glucans. Glucans also form the primary constituent for the exopolysaccharide matrix of the *S. mutans* biofilm.

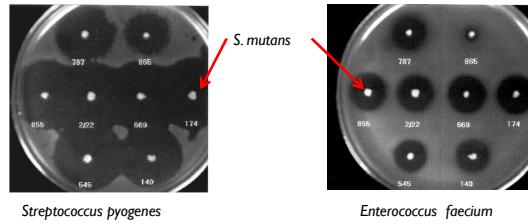
48

Bacteriocins

- Bacteriocins: ribosomally synthesized peptide antibiotics
- Most if not all clinical isolates of *S. mutans* produce *at least* one type of bacteriocin
- The bacteriocins produced by *S. mutans* are referred to as “mutacins”
- Why would *S. mutans* produce mutacins?

49

Bacteriocins



Agar plate showing colonies of *S. mutans* clinical isolates inhibiting the growth of other species. Zones of inhibition are indicative of the presence of mutacin.

S. mutans utilizes weaponry to kill competitors (including foreign pathogens).

50

End of Lecture 1

In the next lecture, we will discuss the major research themes and techniques used to study the concepts we just discussed...

51