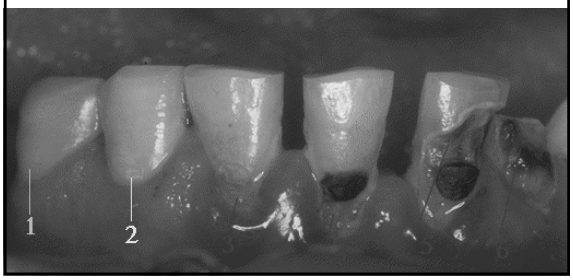
Dental Conference - MID

Dental Caries

October 28, 2004

Dental Caries

Demineralization of the tooth surface caused by bacteria



Chemicoparasitic theory – microbiological basis of dental caries

- Proposed in 1890 by W. D. Miller in his book "The microorganisms of the human mouth" based upon the work done in Robert Koch's laboratory in Berlin
- Acid and parasite
- Showed that the degradation of carbohydrate-containing foods resulted in acid formation and was able to demonstrate this process *in vitro* with isolated oral bacteria and extracted teeth.
- Concluded that dental caries was caused by multiple species of oral bacteria
- No specific bacteria was implicated – "non-specific"

Miller's major conclusion

- Dental caries was caused by multiple species of oral bacteria
- "Non-specific plaque hypothesis".
- Proper prevention is therefore is to remove or minimize multiple bacterial species
  - Practice of tooth brushing, flossing and professional tooth cleaning

Microbial etiology of Dental Caries

- *Mutans Streptococci*
  - Requires a relatively high proportion (2-10%) of *mutans streptococci* within dental plaque.
  - Possess adherence activity (to tooth surface)
  - Produce higher amounts of acid from sugars than other bacterial types, and possess acid tolerance
  - Produce extracellular polysaccharides from sucrose.
- *Lactobacilli*
  - Dentin, root caries, acidogenic, acid tolerant
- *Actinomyces viscosus*
  - Acidogenic and acid tolerant

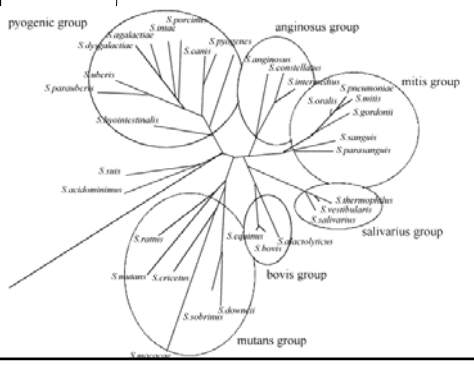
Current diagnosis and treatment

- Future diagnostics using microbiology
  - Detection and monitoring of cariogenic bacteria
  - others
- Potential preventive measures based on microbiological principle
  - Preventing bacteria from colonizing tooth surface
  - Local and topical antimicrobial agents
  - Replacement therapy

Mutans Streptococci

The Mutans Streptococci		
Species	**Serotype**	**Host**
S. mutans	c, e, f	Human
S. Sobrinus	d, g	Human
S. cricetus	a	Human, animal
S. ferus	-	Rat
S. ratti (rattus)	b	Human, rodents
S. macacae	-	Monkey
S. downei	h	Monkey

Classification based on 16S rRNA

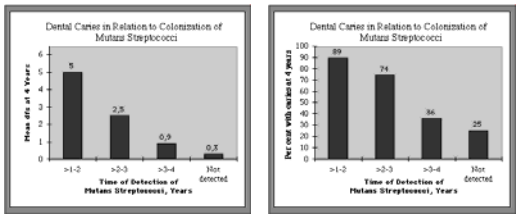


Early acquisition and transmission

- *Mutans streptococci* appear in the mouth after teeth have erupted as they need solid surfaces to colonize.
- The establishment of the bacteria seems rather slow the first two years, and one talks about a "window of infectivity" which would open after about two years.
- There seems to be a family pattern as similar types of bacteria have been found in the mouth of child and parents, in particular mothers.

Mutans Streptococci - prevalence

- Global distribution – found in all populations
- High counts - 10^6/ml saliva
- Usually, serotype c (Streptococcus mutans) is the dominating serotype. One person can have several serotypes (both *Streptococcus mutans* and *Streptococcus sobrinus*).



Lactobacilli

- Gram-positive bacteria which are commonly isolated from the oral cavity.
- Cariogenic, highly acidogenic organisms, and early work implicated lactobacilli in the initiation of dental caries.
- Subsequent research has shown that they are associated more with carious dentine and the advancing front of caries lesions rather than with the initiation of the disease.
- Usually lactobacilli comprise less than 1% of the total cultivable microflora. However, their proportions and prevalence may increase at advanced caries lesions both of the enamel and of the root surface.

Sugar metabolism of cariogenic bacteria

- Acid production (lactate) from glucose and fructose
- Formation of extracellular polysaccharides (glucose polymer, fructose polymer) from the energy of the disaccharide bond of sucrose. (glucosyltransferase, fructosyltransferase)
  - Increase the thickness of plaque substantially
  - Changing the chemical nature of its extracellular space from liquid to gel.
  - The gel limits movement of some ions, protects the plaque biofilm from salivary buffering. Plaque which has not had contact with sucrose is both thinner and better buffered.

The metabolism of S. mutans

- Key to the pathogenesis of dental caries
  - Genome sequence shows that *S. mutans* can metabolize a wider variety of carbohydrates than any other G(+) microorganism
  - The fermentation of these carbohydrates is the principal source of energy for *S. mutans*
  - The glycolytic pathway leads to the production of pyruvate, to lactic acid (by LDH activity), formate, ethanol and acetate
  - The acidic environments are responsible for the damage of tooth structure
  - Acid tolerant – based on a membrane-bound, acid stable, proton-translocating ATPase

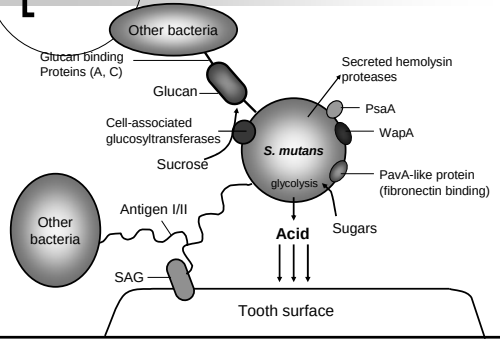
Virulence factors of S. mutans

- Production of acid
- Adhesins
  - Wall-associated protein A (WapA)
  - *S. mutans* LraI operon (SloC)
  - Glucan-binding proteins A and C
- Adherence mechanism

Two methods of attachment

- Sucrose independent –using ionic and lectin like interaction
  - Adhere to salivary agglutinin glycoprotein (SpaP: Streptococcal protein antigen P, *aka* antigen I/II)
    - Isogenic mutants of SpaP
    - Passive immunization study
  - Adhere to other bacteria, the extracellular matrix and epithelial cell-surface receptors
- Sucrose dependent
  - Adhere to tooth surface by synthesizing glucans by glucosyltransferases
  - Glucan promotes cell-cell aggregation by interacting with surface-associated glucan binding protein

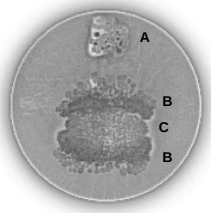
Virulence factors of S. mutans



Kiss Plates – ecological implications

Regions "A" and "B" The bacteria growing here are mostly staphylococci. Most of these will be *Staphylococcus epidermidis*. bright yellow, golden-colored, colonies which will probably be *Staphylococcus aureus*.
On the left side of region "A" above some colonies have produced a clear zone in the agar. This is known as beta-hemolysis.

Region "C" are much smaller than the ones in areas "A" and "B" and are light grey in color. This is typical of streptococci



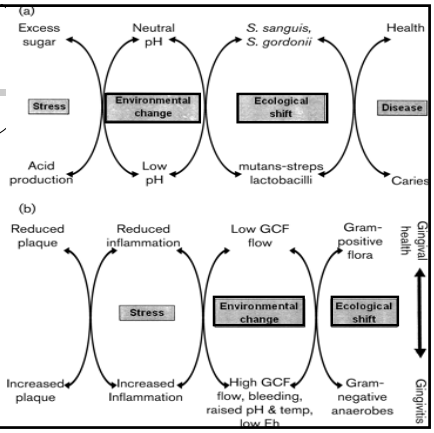
Ecological basis of dental caries

- Environmental changes
  - A variety of environmental signals in complex communities
- Ecological shift
  - The signal triggers adaptation to acid environment
- Biofilm characteristics

Virulence properties of Streptococcus mutans

- Adhesion, acidogenicity, and acid tolerance
- Each of these properties works coordinately to alter dental plaque ecology.
- The ecological changes are characterized by increased proportions of *S. mutans* and other species that are similarly acidogenic and aciduric.
- The selection for a cariogenic flora increases the magnitude of the drop in pH following the fermentation of available carbohydrate and increases the probability of enamel demineralization.

Ecological plaque hypothesis



Replacement Therapy

- Possible life-long cavity protection
- Little or no risk of side effects since the product is a strain of bacteria that occurs naturally in the human body
- Minimal patient education and compliance
- Suitable for use by the general population

Replacement therapy of a bacterial disease

- Replacing a specific bacterial pathogen with a non-pathogenic strain, an effector strain
- An effector strain
  - should not cause disease itself or disrupt the ecosystem to other disease state
  - must persistently colonize the host tissue at risk and thereby prevent colonization or outgrowth of the pathogen
  - should possess a high degree of genetic stability

Replacement therapy for the prevention of dental caries

- Lactate dehydrogenase (LDH)-deficient mutants of *Streptococcus rattus* were shown to have little or no cariogenic potential *in vitro* and in various rodent models.
- A naturally occurring strain (JH1000) of *Streptococcus mutans* was isolated that produces a lantibiotic called mutacin 1140 capable of killing virtually all other strains of *mutans streptococci* against which it was tested.

Construction of lactate dehydrogenase deficient mutant

- Deleting virtually all the *ldh* open reading frame in JH1140 (mutacin producing, supercolonizing strain,)
- Subsitituting the *ldh* ORF with the *adh*B ORF from *Zymomonas mobilis*
- The resuting clone BCS3-L1
  - No detectable lactic acid production
  - Less total acid production due to increased production of ethanol and acetoin
  - Less cariogenic that JH1140 in both gnotobiotic and conventional-rodent model
  - Strong colonization potential