Dental conference III

Periodontal disease

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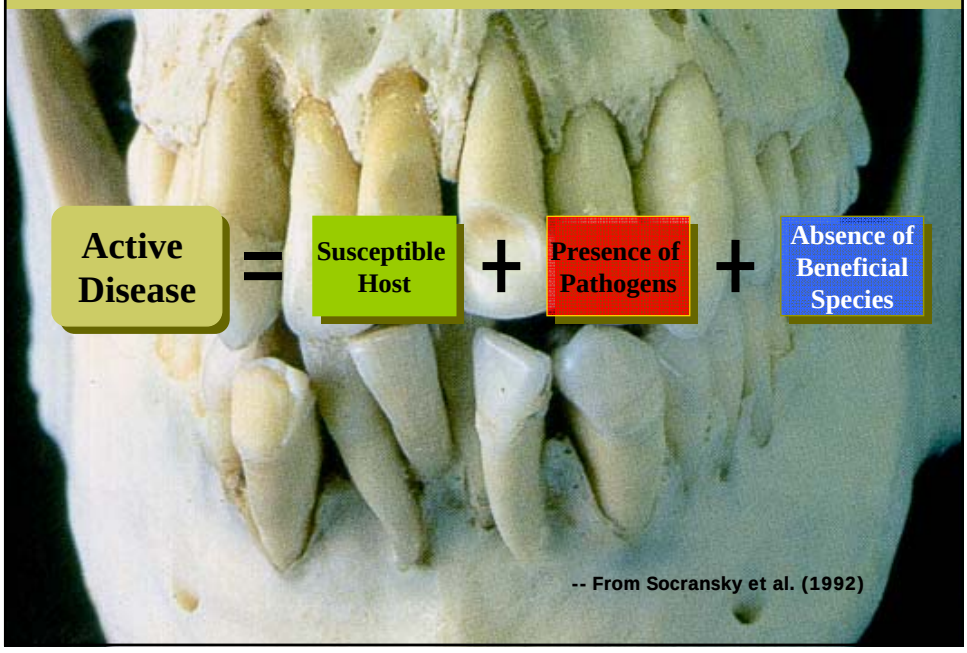
Destructive periodontal disease



Dental plaque biofilm infection

- Ecological point of view
  - Ecological community evolved for survival as a whole
  - Complex community of more than 400 bacterial species
- Dynamic equilibrium between bacteria and a host defense
  - Adopted survival strategies favoring growth in plaque
  - "Selection" of "pathogenic" bacteria among microbial community
    - Selection pressure coupled to environmental changes
  - Disturbed equilibrium leading to pathology
  - Opportunistic infection

Difficulties in defining Periodontal Pathogens

- Classical Koch's Postulate
  - designed for monoinfections
- Technical difficulties
- Conceptual problems
- Data analysis

From Socransky *et al*. J. Clin Periodontol, 14:588-593, 1987

100 Years of Periodontal Microbiology

1890 — **Specific**

Fusoformis fusiformis (1890)
Streptococci (1906)
Spirochetes (1912)
Amoeba (1915)

1930 — **Non-specific**

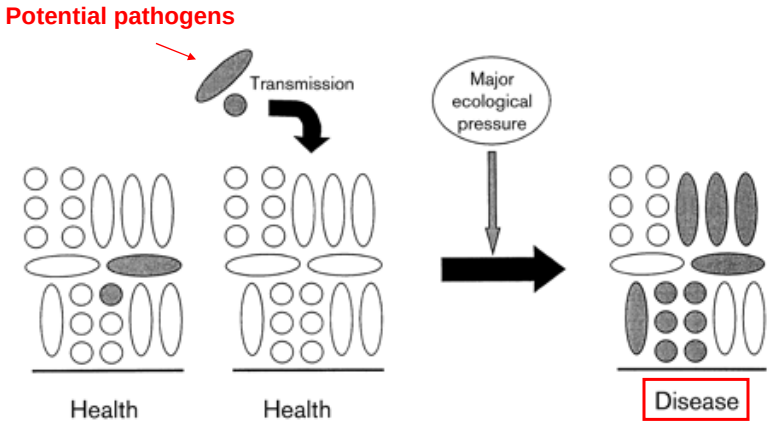
Mixed Infection - Fusospirochetal (1930)
Mixed Infection - with Black pigmented *Bacteroides* (1955)

1970 — **Specific**

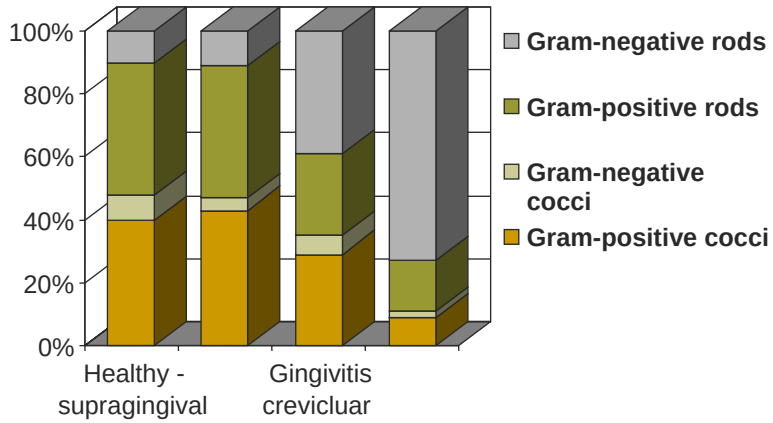
Spirochete - ANUG (1965)
A. viscosus (1969)
A. actinomycetemcomitans (1976)
P. gingivalis (1980)
P. intermedia (1980)
T. denticola
T. forsythia (B. forsythus)

1990

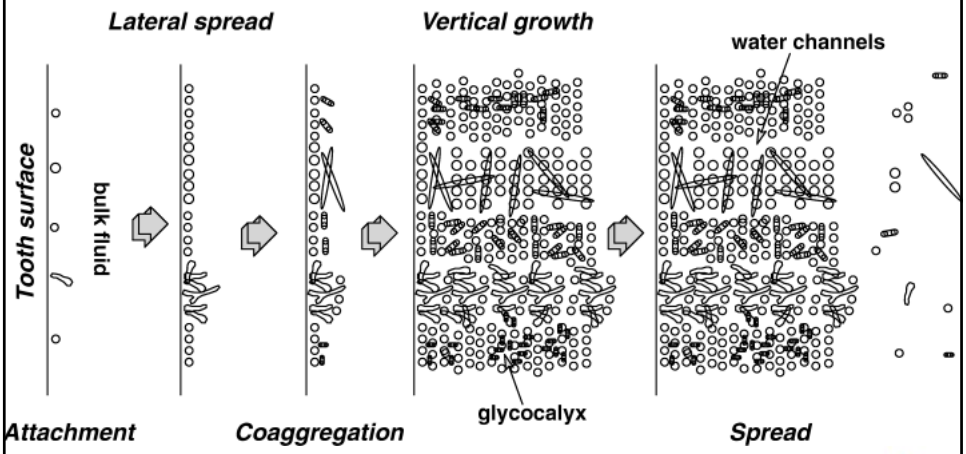
Health vs. disease microflora in dental plaque

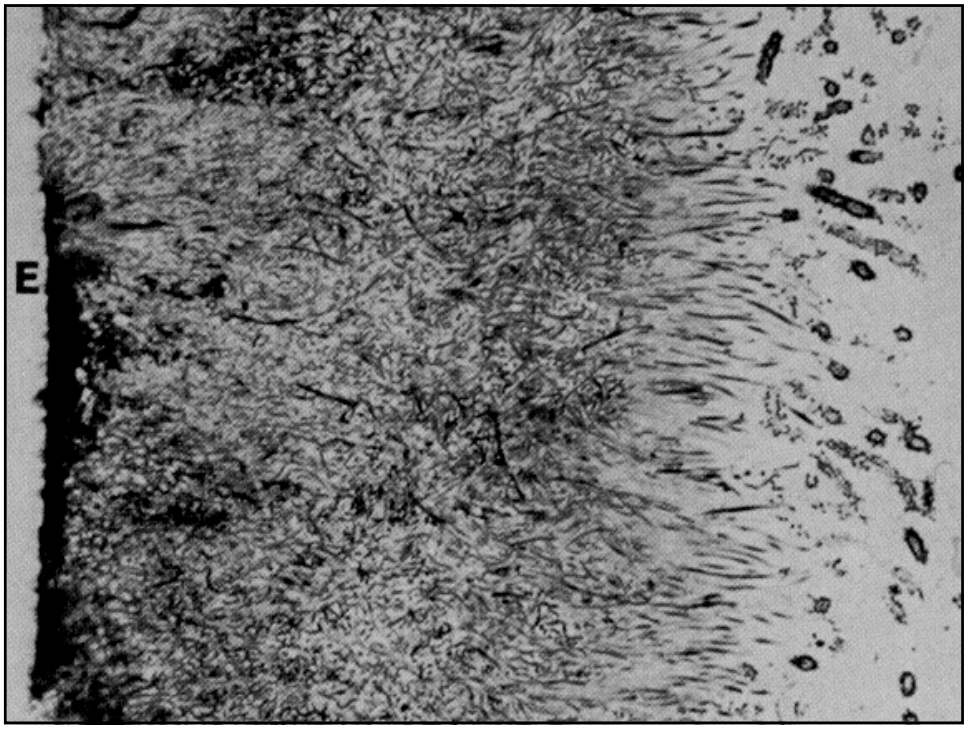


Microbiota Associated with Periodontal health, Gingivitis, and Advanced periodontal disease



Development of dental plaque biofilm

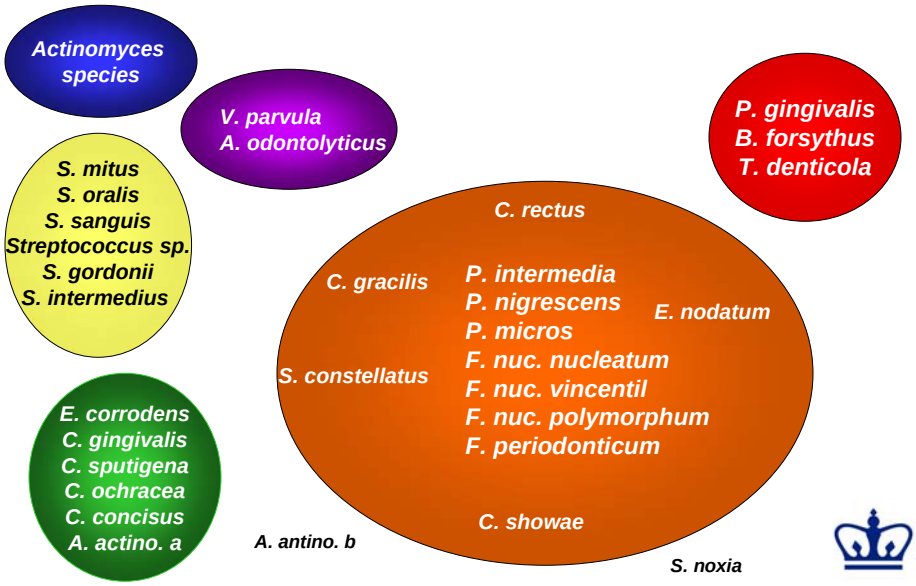




Microbial complexes in biofilms

- Not randomly exist, rather as specific associations among bacterial species
- Socransky et al. (1998) examined over 13,000 subgingival plaque samples from 185 adults, and identified **six** specific microbial groups of bacterial species

Subgingival microbial complex



Criteria for defining putative periodontal pathogens

- Association with disease
- Elimination should result in clinical improvement
- Host response to pathogens
- Virulence factors
- Animal studies demonstrating tissue destruction

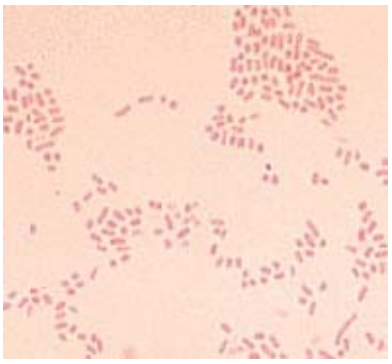
Possible etiologic agents of periodontal disease

- ***Actinobacillus actinomycetemcomitans***
- ***Porphyromonas gingivalis***
- ***Tannerella forsythia (Bacteroides forsythus)***
- ***Treponema denticola***
- *Prevotella intermedia*
- *Fusobacterium nucleatum*
- *Eikenella corrodens*
- *Campylobacter rectus* (*Wolinella recta*)
- *Peptostreptococcus micros*
- *Streptococcus intermedius*

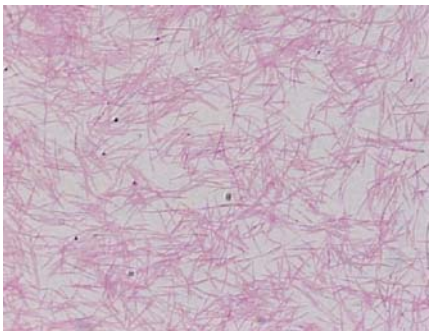
Actinobacillus actinomycetemcomitans

- First recognized as a possible periodontal pathogen in LJP (Newman et al., 1976)
- Majority of LJP patients have high Ab titers against *Aa*
- Successful therapy lead to elimination or significant decrease of the species
- Potential virulence factors; leukotoxin, cytolethal distending toxin, invasion, apoptosis
- Induce disease in experimental animals
- Eleveated in "active lesions", compared with non-progressing sites
- Virulent clonal type of *Aa*
  - LJP patients exhibit specific RFLP pattern, while healthy pts exhibit other patterns
  - Increased leukotoxin production by Aa strains isolated from families of African origin, a 530 bp deletion in the promoter of the leukotoxin gene operon
    - 22.5 X more likely to convert to LJP than who had Aa strains with the full length leukotoxin promoter region
- Associated with refractory periodontitis in adult patients

Phenotypes – gram stain



A. actinomycetemcomitans



F. nucleatum

Porphyromonas gingivalis

- Gram (-), anaerobic, asaccharolytic, black-pigmented bacterium
- Suspected periodontopathic microorganism
  - Association
    - Elevated in periodontal lesions, rare in health
    - Elimination or suppression resulted in successful therapy
  - Immunological correlation
    - Elevated systemic and local antibody in periodontitis
  - Animal pathogenicity
    - Monkey, dog, and rodent models
  - Putative virulent factors

Spirochetes

- G (-), anaerobic, spiral, highly motile
- ANUG
- Increased numbers in deep periodontal pockets
- Difficulty in distinguishing individual species
  - 15 subgingival spirochetes described
  - Obscure classification - Small, medium, or large
- *T. denticola*
  - More common in diseased, subgingival site
- Uncultivated "pathogen-related oral spirochetes
  - Detected by Ab cross-reactivity to *T. pallidum* antibody

Prevotella intermedia/Prevotella nigrescens

- Strains of "*P. intermedia*" separated into two species, *P. intermedia* and *P. nigrescins*
- Hemagglutination activity
- Adherence activity
- Induce alveolar bone loss
- In certain forms of periodontitis
- Successful therapy leads to decrease in *P. intermedia*

Fusobacterium nucleatum

- G(-), anaerobic, spindle-shaped rod
- Has been recognized as part of the subgingival microbiota for over 100 years
- The most common isolate found in cultural studies of subgingival plaque samples:7-10% of total isolates
- Prevalent in subjects with periodontitis and periodontal abscess
- Invasion of epithelial cell
- Apoptosis activity

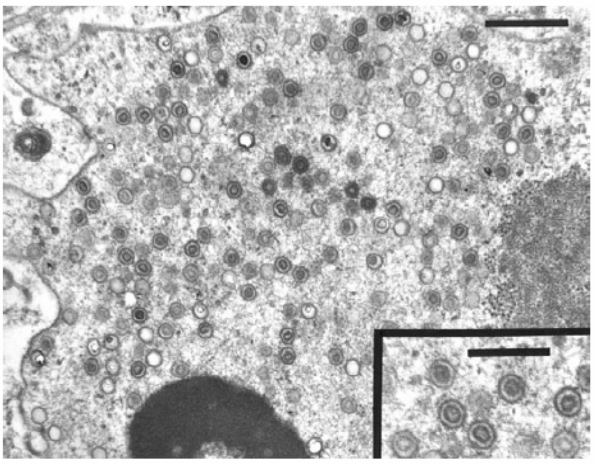
Other species

- *Campylobacter rectus*
  - Produce leukotoxin
  - Contains the S-layer
  - Stimulate gingival fibroblast to produce IL-6 and IL-8
- *Eikenella corrodens*
- *Peptostreptococcus micros*
  - G(+), anaerobic, small asaccharolytic
  - Long been associated with mixed anaerobic infections
- *Selemonas* species
  - Curved shape, tumbling motility
  - *S. noxia* found in deep pockets, conversion from healthy to disease site
- *Eubacterium* specues
- The “milleri” streptococci
  - *S. anginosus, S. constellatus, S. intermedius*

Virus and periodontal disease

- Involvement of herpesvirus (human cytomegalovirus, HCMV and Epstein-Barr virus, EBV)
  - Genomes of HCMV and EBV occur at high frequency in aggressive, HIV-associated, ANUG, and advanced type periodontitis associated with medical disorders
- HCMV infects periodontal monocytes/macrophages and lymphocytes, and EBV infects periodontal B-lymphocytes
- Herpesvirus-infected inflammatory cells may
  - Elicit tissue-destroying cytokines
  - Exert diminished ability to defend against bacterial challenge

Herpesvirus-like virions



Gingival epithelial cells of HIV-associated necrotizing ulcerative periodontitis.

Microbial pathogenicity

- Pathogenicity
  - The likelihood of causing disease
- Virulence
  - A quantitative measure of pathogenicity
  - Virulent, avirulent strain
- Virulence factors
  - Gene products that enhance a microorganism's potential to cause disease
  - Virulence genes
- "the pathogenic personality" of a specific pathogen

Virulence factors

- Gene products that enhance a microorganism's potential to cause disease
- Involved in all steps of pathogenicity
  - Attach to or enter host tissue
  - Evade host responses
  - Proliferate
  - Damage the host
  - Transmit itself to new hosts
- Virulence genes

Expression of virulence factors

- Constitutive
- Under specific environmental signals
  - Can be identified by mimicking environmental signals in the laboratory
  - Many virulence-associated genes are coordinately regulated by environmental signals
- Only *in vivo*
  - Cannot be identified in the laboratory
  - Anthrax toxin, cholera toxin

Identifying virulence factors

- Microbiological and biochemical studies
  - *In vitro* isolation and characterization
  - *In vivo* systems
- Genetic studies
  - Study of genes involved in virulence
  - Genetic transmission system
  - Recombinant DNA technology
    - Isogenic mutants
    - Molecular form of Koch's postulates (Falkow)

Virulence factors of *A. actinomycemtemcomitans*

- Leukotoxin (RTX)
  - Induce apoptosis
- Cytolethal distending toxin (CDT)
- Chaperonin 60
- LPS
  - Apoptosis, bone resorption, etc
- OMP, vesicles
- Fimbriae
- Actinobacillin
- Collagenase
- Immunosuppressive factor

Virulence factors of *P. gingivalis*

- Involved in colonization and attachment
  - Fimbriae, hemagglutinins, OMPs, and vesicles
- Involved in evading (modulating) host responses
  - Ig and complement proteases, LPS, capsule, other antiphagocytic products
- Involved in multiplying
  - Proteinases, hemolysins
- Involved in damaging host tissues and spreading
  - Proteinases (Arg-, Lys-gingipains), Collagenase, trypsin-like activity, fibrinolytic , keratinolytic, and other hydrolytic activities

An Example of Studying Microbial Pathogenesis

Hypothesis

S-layer of *T. forsythia* is a virulence factor

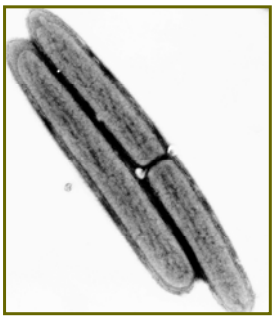
Tannerella forsythia (formerly B. forsythus)

- *T. forsythia* is a gram-negative, filament-shaped, non-motile, non-pigmented oral bacterium
- *T. forsythia* has been associated with advanced and recurrent periodontitis
- Implicated as one of three strong candidates for etiologic agents of periodontal disease
  - *Actinobacillus actinomycemtemcomitans*
  - *Porphyromonas gingivalis*
  - *Tannerella forsythia*
- One of "red complex" pathogenic bacteria

Morphology of *T. forsythia*

Colony

Gram stain

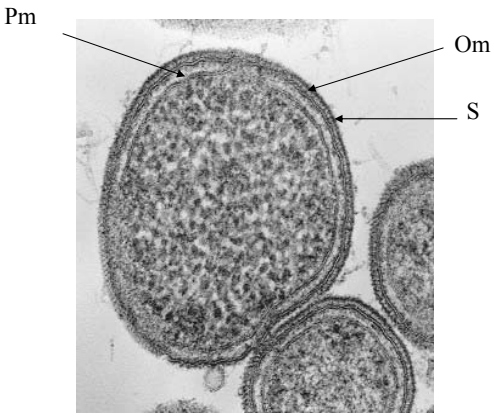


EM Negative staining

Virulence factors of *T. forsythia*

- Pathogenicity is virtually unknown
  - Little information on virulence factors
  - Fastidious nature of microorganisms
- Putative Virulence factors
  - Proteolytic enzymes, trypsin-like enzymes
  - Sialidase (Neuraminidase)
  - Leucin-rich surface protein (BspA)
    - BspA isogenic mutant
    - Adhesin, inducing alveolar bone loss (mice)
  - Surface (S-) layer ?

Surface layer of *T. forsythia*



Thin section of *T. forsythia*. S: S-layer; Om: outer membrane; Pm: plasma membrane

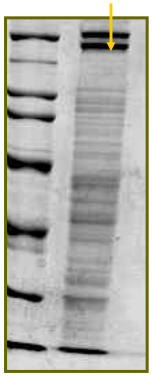
Identification of the genes responsible for causing disease

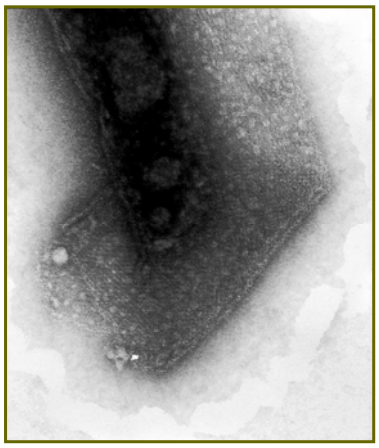
- A Molecular form of Koch's postulates
  - The phenotype should be associated with pathogenic species (strains)
  - Specific inactivation of genes associated with virulence should lead to a decrease in virulence
  - Complementing inactivated genes with the wild-type genes should restore full virulence

Falkow, 1988

Isolation of S-layer from *T. forsythia*

Most abundant cellular proteins

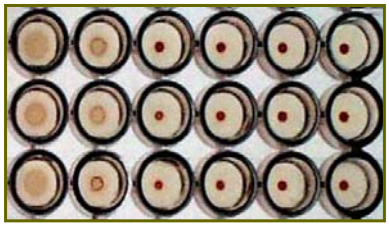




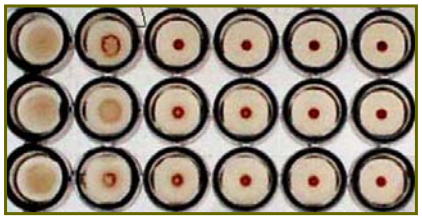
Negative staining

Hemagglutination activity of *T. forsythia*

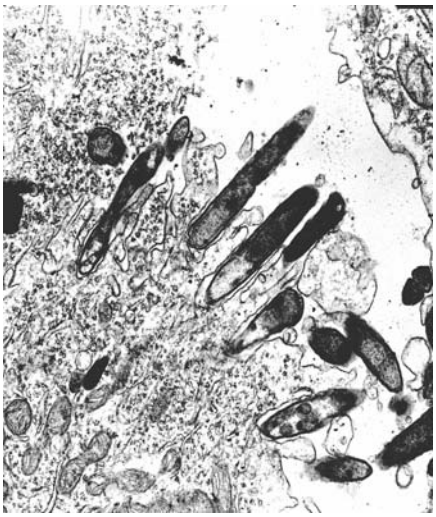
Whole cell

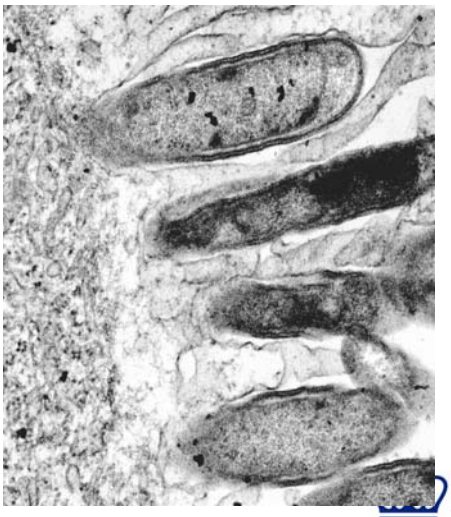


Isolated S-layer

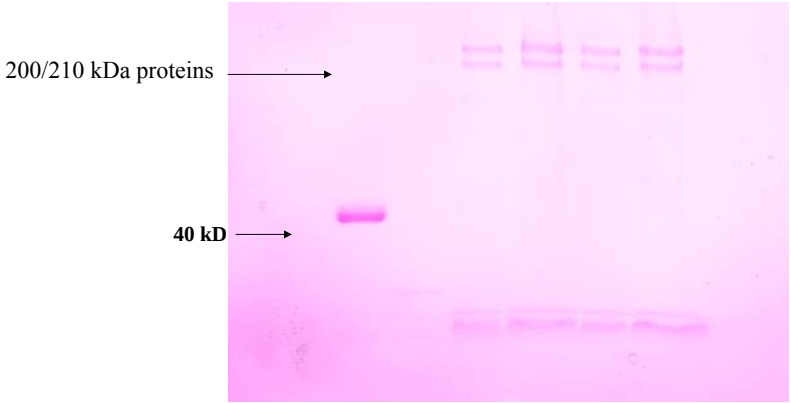


T. forsythia adheres to KB cells

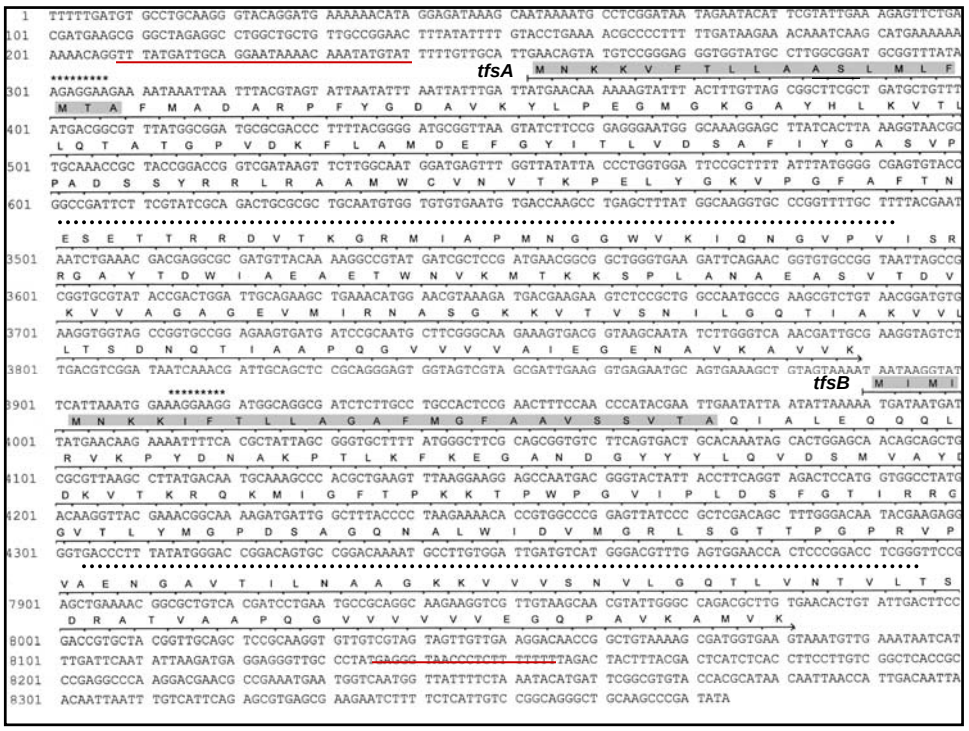


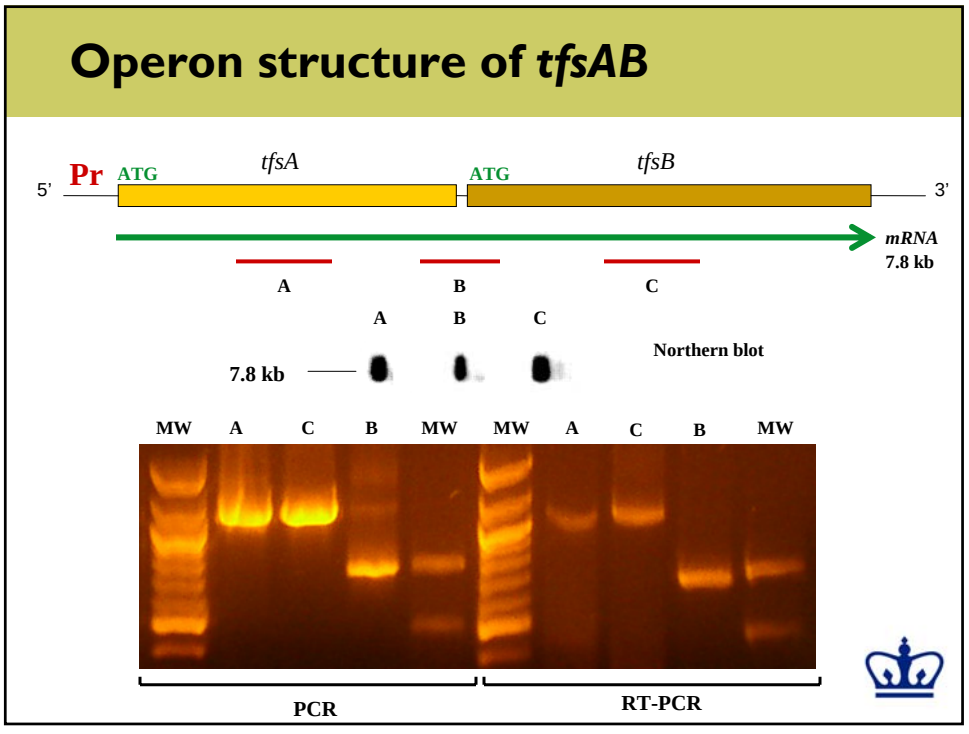


S-layer proteins are glycosylated



Carbohydrate components are oxidated by periodate and stained


tfsA
tfsB


Operon structure of tfsAB
Pr
ATG
tfsA
ATG
tfsB
5'
3'
mRNA
7.8 kb
A
B
C
A
B
C
7.8 kb
Northern blot
MW
A
C
B
MW
MW
A
C
B
MW
PCR
RT-PCR

Confirming S-layer as a virulence factor

- Construction of isogenic mutants lacking S-layer
- Use of relevant animal model for periodontal disease in testing virulence/pathogenicity