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First Time in Human Safety Study of *Streptococcus mutans* Lactic Acid-deficient Effector Strain (A2JM) Administered in Conjunction with Twice Daily Dose of D-alanine Mouthwash in Healthy Adult Male Subjects for Replacement Therapy as an Aid in the Protection Against Dental Caries

Protocol #0311-614

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Program Team

- Principal Investigator: Constance Stone, DMD
- Co-investigators: Jeffrey D. Hillman, DMD, PhD
  Robert Zahradnik, PhD
  Eric Chojnicki, PhD, MBA

Consultants: Michael Rosenberg, MD, MPH
Dennis Cvitkovitch, PhD
Mark Herzberg, PhD
R. Michael Blaese, MD

- Corporate Sponsor: Oragenics, Inc.

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Significance

- Dental caries is the most common chronic disease (5 billion people worldwide)
- Approximately $40 billion was spent in the U.S. in 2003 on dental caries (5% of total health care costs)
- An increasing body of evidence has associated oral infections with systemic diseases, such as cardiovascular disease

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Background

- An infectious disease, principally caused by the indigenous plaque bacterium *S. mutans*
- Dietary sugar is metabolized by *S. mutans* to produce lactic acid, which dissolves the mineral portion of tooth enamel and dentine
- Current methods for prevention aim at reducing the levels of *S. mutans* or making the teeth more resistant to acid attack
  - All require patient compliance

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Hypothesis

- The presence of a particular microorganism can prevent colonization by a pathogen or keep its numbers below the level required for it to manifest disease.
- This hypothesis forms the basis for an approach to prevent infections called *replacement therapy*.

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Replacement Therapy

- Depends on finding or creating an *effector strain* that has 3 essential properties:
  1. It does not cause disease itself
  2. It can persistently colonize the tissue at risk and outcompete disease-causing strains
  3. It is genetically stable

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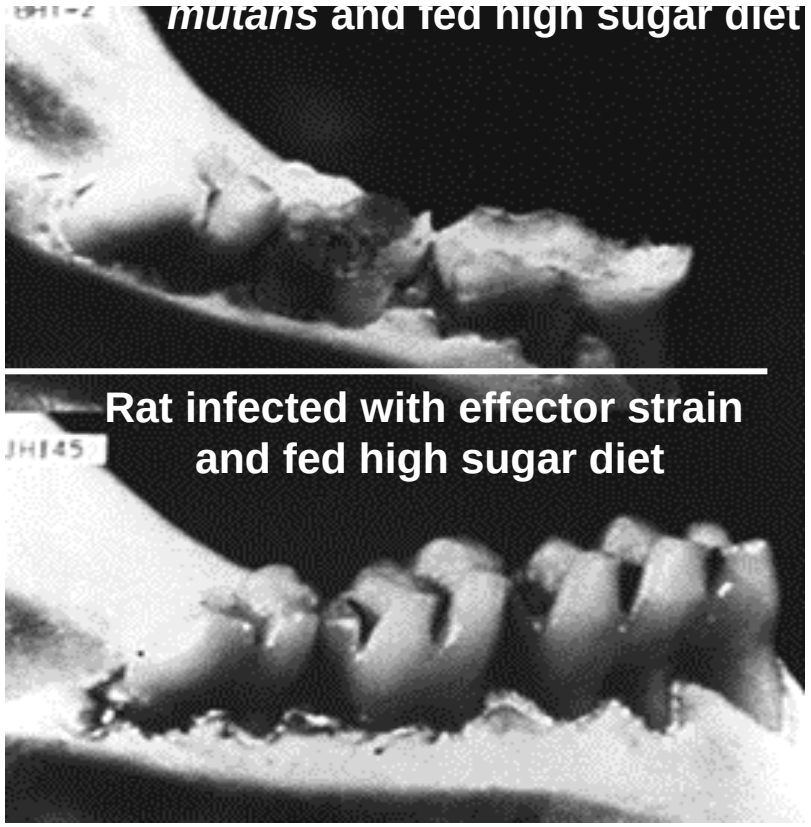
The A2JM Effector Strain: Pathogenic Potential

- The gene for lactate dehydrogenase (*ldh*) was deleted
- Deletion of *ldh* killed the bacteria; compensate for this by adding a supplemental gene for alcohol dehydrogenase (*adhB*)



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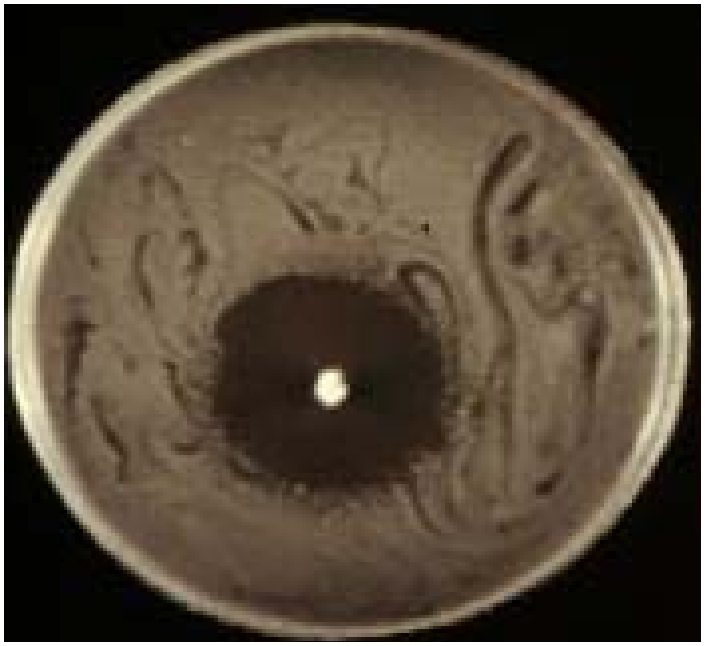
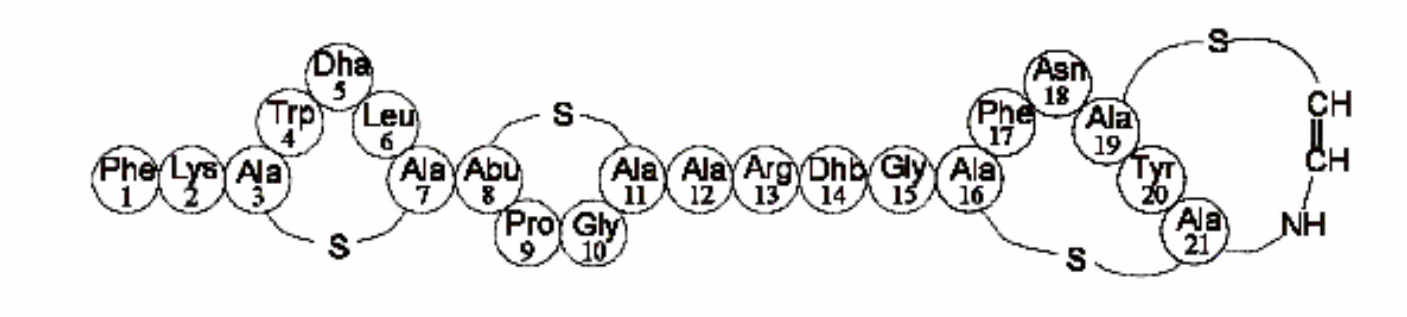
The A2JM Effector Strain: Colonization Potential

- Perform strain construction starting with a human *S. mutans* isolate that naturally produces a bacteriocin capable of killing all other *S. mutans* strains





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The A2JM Effector Strain: Genetic Stability

- LDH deficiency due to a deletion mutation
- Genetic transformation occurs at very low frequencies due to:
  - A naturally occurring insertion mutation in the competence gene, *comC*, which reduces transformation frequency
  - An engineered deletion mutation in another competence gene
- No prophage or known transducing phage

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Horizontal Transmission

- Transmission is typically vertical (mother to child)
- The minimal infectious dose for A2JM is 1000-fold higher than for its *ldh*$^{+}$ parent
- Adult male volunteers colonized with mutacin 1140-producing *S. mutans* did not transmit this strain to spouses or children over a 15-year period

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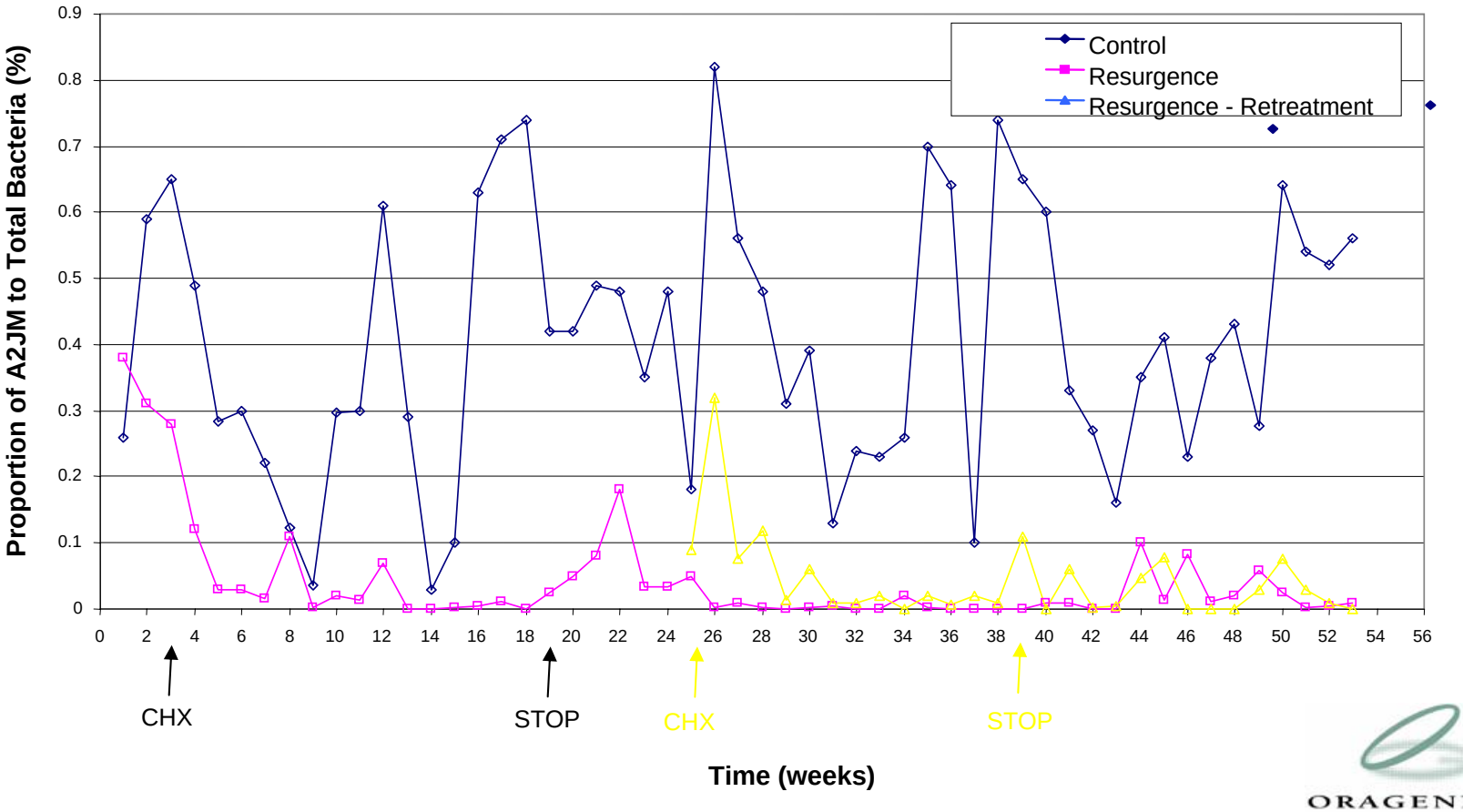
Additional Safeguard

- A2JM is dependent on environmental D-alanine due to a deletion mutation in alanine racemase (*dal*)



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Laboratory and Animal Testing of A2JM

- It did not cause significant tooth decay in rats
- It persistently colonized the teeth of rats
- It displaced other *S. mutans* strains
- It was genetically stable in the laboratory and in animals
- It showed no toxicity in acute and chronic (1 year) tests
- It did not disrupt the normal oral flora

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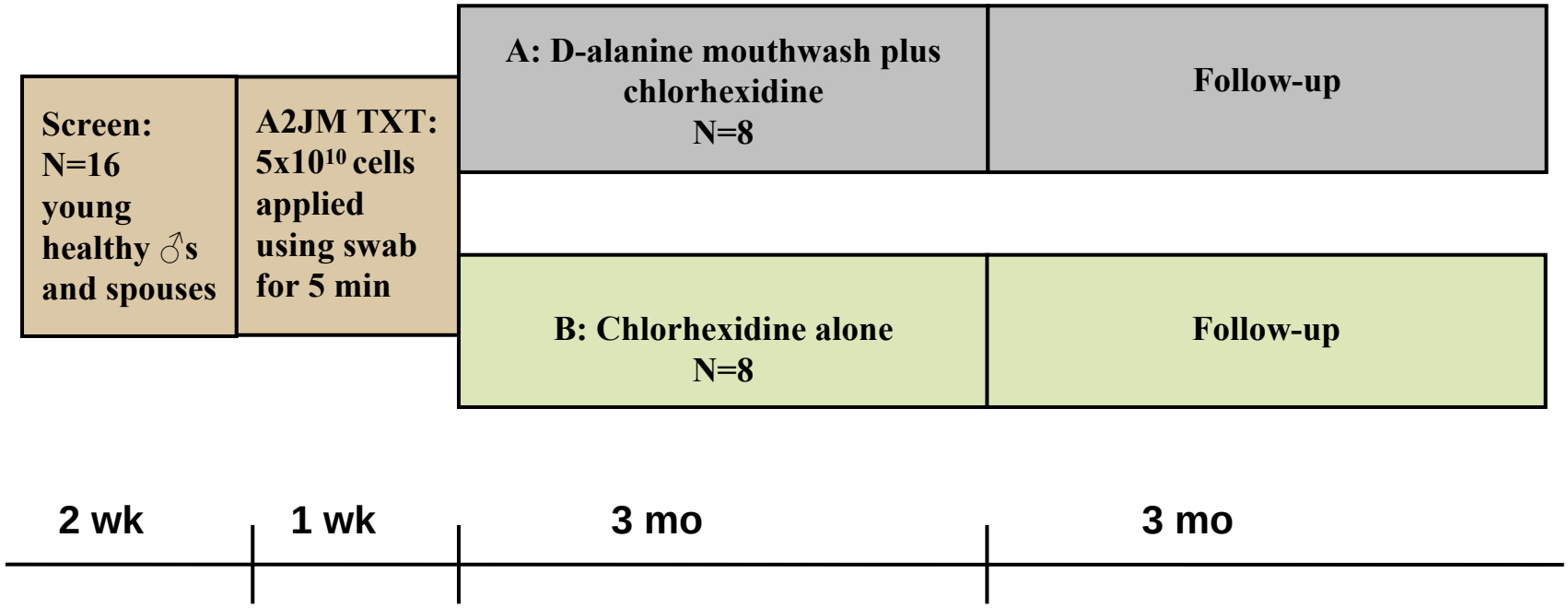
Study Design Scheme



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Objectives

- Primary Objective
  - To assess safety and tolerability of A2JM and D-alanine
- Secondary Objectives
  - To estimate stability of A2JM genetic profile
  - To estimate A2JM transfer to partners
  - To determine if A2JM can be eradicated or reduced in numbers below its minimal pathogenic dose

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Inclusion Criteria

- Subjects must be male between 21 and 35 years old
- Subject must be in a stable, monogamous relationship
- Both partners must be healthy
- No less than 20 natural, minimally restored teeth
- Partner must remain non-pregnant throughout the trial
- Both subject and partner must have an indigenous mutans streptococcus strain

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Exclusion Criteria

- Abnornmal baseline physiological findings
- Rheumatic fever, valvular heart disease, SBE
- Oral abnormalities that compromise gingival intergrity
- Significant chronic clinical illness (e.g., hepatitis, HIV)
- Children living in the same household
- Employed as food handlers, day care or healthcare provider

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Outcome Measures

- Measure frequency and nature of adverse events
- Collect saliva samples from subjects and spouses
  - Determine genetic stability
  - Determine occurrence of horizontal transmission

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Advantages of Replacement Therapy

- Lifelong protection from a single treatment
- No patient compliance required

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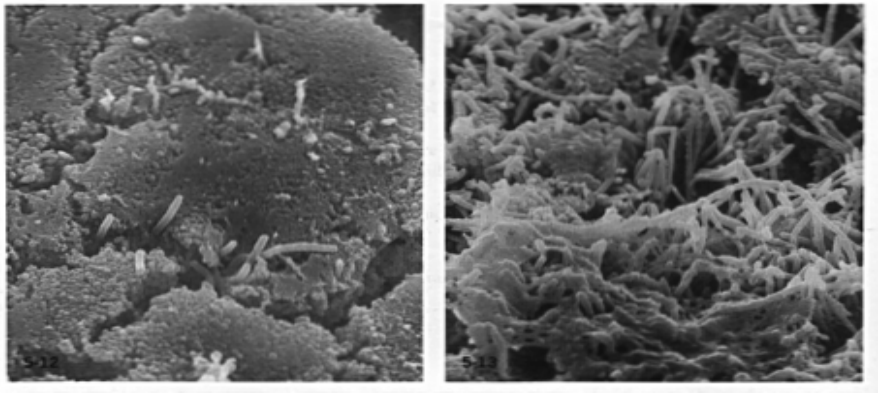
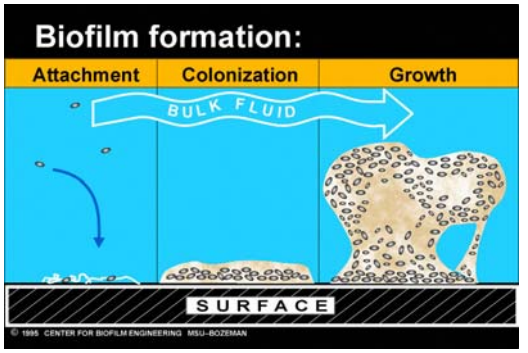
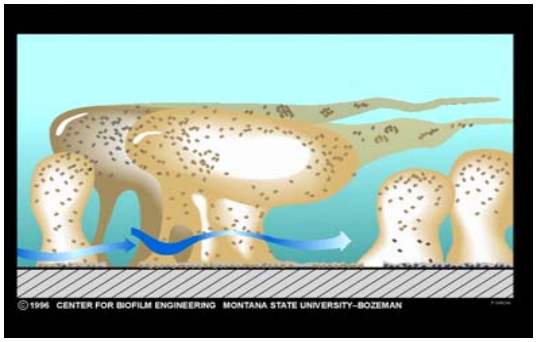
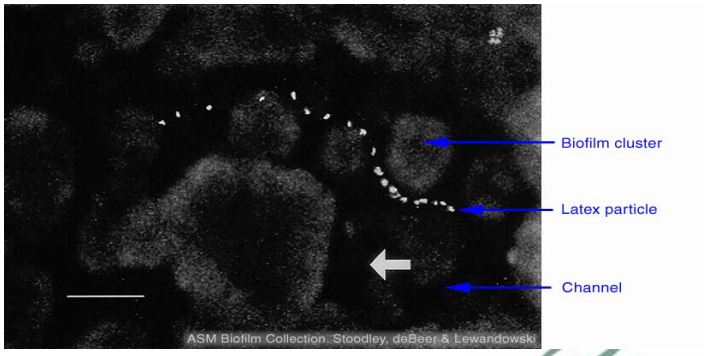
Plaque is a Complex Biofilm









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