CLINICAL REVIEW

Oral Lactobacilli and Dental Caries: A Model for Niche Adaptation in Humans

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Appendix

Study Design and Methods

To measure and characterize the diversity and abundance of lactobacilli in saliva and dental plaque, we recruited 2 matched-pair cohorts of children; the first were caries free and the second had severe early childhood caries (S-ECC). Detailed methodology and selection criteria are presented in a 2015 open access paper (Li et al. 2015). To facilitate reading of this review, we include here a brief summary of the study. A total of 76 children, 3 to 6 y of age, comprised the study sample of 38 diagnosed with S-ECC and 38 age- and gender-matched caries-free (CF) children. Both groups having similar demographic profiles were recruited from the Pediatric Dental Clinic at the Bellevue Hospital in New York City. The study was conducted at the New York University College of Dentistry and the Pediatric Dental Clinic of Bellevue Hospital Center. Institutional review board approvals were obtained from the New York University School of Medicine and the New York City Health and Hospital Corporation (for Bellevue Hospital Center). Informed consent was obtained from the parents or legal guardians before enrollment of children in the study.

The inclusion criteria for this study were based on the American Academy for Pediatric Dentistry definition for S-ECC (American Academy of Pediatric Dentistry 2007). More specifically, S-ECC was minimally defined as caries in children younger than 3 y with any sign of smooth-surface caries (noncavitated or cavitated lesions, decayed-missing-filled surfaces [dmfs]). Because every child with S-ECC experienced caries in excess of 10 or more decayed-filled surfaces coupled with their young age, they were treated under general anesthesia at Bellevue Hospital. Children who were caries free (CF) were defined as having teeth that showed no evidence of decay, treated or untreated, on any surfaces (Dye et al. 2007). To be eligible for this study, children were 3 to 6 y old, were healthy without chronic diseases other than dental caries, and had not taken antibiotics within 3 mo prior to the bacterial sample collection.

A pooled sample of nonstimulated saliva plus supra-gingival plaque was collected from each child, dispersed, diluted, and cultivated on LBS selective medium (Becton Dickinson, Sparks, MD, USA) for total lactobacilli recovery. Duplicate samples were incubated anaerobically at 30 °C and 37 °C for 4 d. In addition, we sampled fissures from children with CF and caries lesions from those with S-ECC. The lactobacilli screening and identification include randomly selecting 50 colonies per plate, extracting DNA from each colony, and genotyping by arbitrarily primed polymerase chain reaction (AP-PCR) (Li and Caufield 1998). Each unique lactobacilli AP-PCR genotype was selected for initial taxonomic assessment based on partial 16S rRNA gene sequencing analysis. After the initial screening, high-quality DNA was obtained from unique lactobacilli isolates and final *Lactobacillus* species identification was confirmed based on the full-length 16S rRNA sequence.

Appendix References

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