

2215 Development of a Novel Oligonucleotide Probe for Detecting *Actinomyces naeslundii*

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Objectives: *Actinomyces naeslundii* (*A. naeslundii*) is a common bacterium associated with periodontal diseases and, occlusal and root surface caries. As the accuracy of biochemical identification is easily affected by both the biochemical and physiological similarity shared by different *Actinomyces* species, and the unstability of biochemical properties in some species, using a probe of DNA sequence of 16S rRNA to detect *A. naeslundii* appears feasible, as the latter is highly conserved. The aim of this study therefore was to develop a species-specific DNA oligonucleotide probe complementary to 16S rRNA of *A. naeslundii*.

Methods: A pair of primers (the forward primer: 5gggtgagtaacacgtgagtaa3; the reverse primer: 5cgagctgacgacaacat3) and one oligonucleotide probe of 17 bases with a tail of 20 thymidines (5TTTTTTTTTTTTTTTTTTTTCACCAAAAAGGCGCCA3) were designed using Webseq and Primer3 software, and synthesized for hybridization. This pair of primers could amplify a specific fragment of the DNA sequence of 16S rRNA gene of *A. naeslundii*, which included a highly variable region. A total of 20 strains of *Actinomyces* spp., comprising *A. naeslundii* (6), *A. viscosus* (3), *A. israelii* (5), *A. bovis* (2), *A. meyeri* (2), and *A. odontolyticus* (2) were used to test specificity of the probe. Polymerase chain reaction (PCR) products of these 20 strains were then amplified and labeled with digoxigenin with the help of the PCR DIG Labeling Mix (Roche, Germany). A DNA-DNA hybridization assay was undertaken using the MINISLOT™ 10 and MINIBLOTTER 28SL system (Immunetics USA).

Results: This oligonucleotide probe clearly detected the six strains of *A. naeslundii* from the pool of 20 strains of different *Actinomyces* spp. without non-specific hybrids.

Conclusions: These results indicate the 17-base oligonucleotide (5CACCAAAAAGGCGCCA3) complementary to the region, positions 94 to 100 (Genebank accession: M33911), can be used as a specific probe to detect *A. naeslundii*.

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[Seq #203 - Candida II](#)

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