Ammonia production from urea by ureolytic oral bacteria is believed to have a significant impact on oral health and the ecological balance of oral microbial populations. In this study we cloned and characterized the urease gene cluster of Actinomyces naeslundii, which is one of the pioneer organisms in the oral cavity and a significant constituent of supragingival and subgingival dental plaque in children and adults. An internal fragment of the ureC gene of A. naeslundii WVU45 was initially amplified by PCR with degenerate primers derived from conserved amino acid sequences of the large catalytic subunit of urease in bacteria and plants. The PCR product was then used as a probe to identify recombinant bacteriophages carrying the A. naeslundii urease gene cluster and roughly 30 kbp of flanking DNA. Nucleotide sequence analysis demonstrated that the gene cluster comprised of seven contiguously arranged open reading frames with significant homologies at the protein and nucleotide sequence levels to the ureABCEFGD genes from other organisms. By using primer extension, a putative transcription initiation site was mapped at 66 bases 5' to the start codon of ureR. A urease-deficient strain was constructed by insertion of a kanamycin resistance determinant within the ureC gene via allelic replacement. In contrast to the wild-type organism, the isogenic mutant was unable to grow in a semidefined medium supplemented with urea as the nitrogen source and was not protected by the addition of urea against killing in moderately acidic environments. These data indicated that urea can be effectively utilized as a nitrogen source by A. naeslundii via a urease-dependent pathway and that ureolysis can prevent A. naeslundii against environmental acidification at physiologically relevant pH values. Therefore, urease could confer to A. naeslundii critical selective advantages over nonureolytic organisms in dental plaque, constituting an important determinant of plaque ecology.

Ureeses are nickel-containing, multisubunit enzymes that catalyze the hydrolysis of urea to ammonia and carbon dioxide, with a net increase in environmental pH. They are highly conserved proteins found in a number of plants, bacteria, fungi, and algae (45). In prokaryotes, urea hydrolysis can confer protection against killing in acidic environments (59) or can provide ammonia, which is a preferred nitrogen source for many bacteria (17). There is also evidence that some organisms, such as Ureaplasma ureolyticum (62) and some alkalo- philes (32), may use ureolysis to generate a proton motive force that can drive ATP synthesis. Expression of a catalytically active urease in bacteria is usually directed by at least seven genes, which in general are arranged in operons (16, 46). Three of the genes encode the structural subunits of urease: the large catalytic subunit (α), encoded by the ureC gene, and the two smaller subunits (β and γ), which are the products of ureB and ureA, respectively. The three subunits can associate in an (αβγ)2 stoichiometry to form the urease apoenzyme (31). Activation of the apoenzyme involves the incorporation of six nickel ions per active trimeric molecule and is accomplished by the coordinated action of four accessory proteins, encoded by ureD, -E, -F, and -G (39, 48). The roles of each of these proteins in the urease holoenzyme assembly process are beginning to be elucidated. A current model for urease apoenzyme activation (52) proposes that UreD functions as a molecular chaperone which maintains the apoenzyme in a competent state, able to receive nickel ions from the nickel donor, UreE. In this model, UreG, which has an identifiable ATP- and GTP-binding domain, participates in some energy-dependent stage of urease activation (47, 67). Additional urease-associated genes, involved in either the regulation of urease expression (ureR) (21, 50) or the transport of nickel into the cell (nixA and ureH) (37, 44), have also been described for ureolytic organisms.

Bacterial urease activity can contribute to the development of several pathologic conditions in humans, such as gastritis (9), pylonephritis (49), and urinary tract stone formation (42). Substantial amounts of urea are also present in human oral secretions, such as saliva and gingival crevicular fluid (28), and a number of indirect observations have suggested an involvement of ureolysis in the pathogenesis of oral diseases (3, 25, 28, 43, 54, 70). Ureolytic activity in supragingival dental plaque can counteract the effects of microbial glycolytic activity and thus prevent plaque acidification (15, 34, 58). By helping to maintain plaque pH at neutral levels, ureolysis could inhibit demineralization of dental enamel, which otherwise occurs in acidic environments (23), and may also prevent ecological shifts in dental plaque commonly associated with caries development (10). For these reasons, alkali generation via ureolysis in the oral cavity can potentially be an important inhibitor of dental caries formation (34, 54). Conversely, the production of ammonia by ureolytic organisms in subgingival plaque may have detrimental effects on periodontal tissues. These include promotion of the precipitation of normally soluble ions from saliva and gingival crevicular fluid, which is induced by alkaline pH and which can lead to the formation of subgingival calculus (25, 38), as well as possible contributions to inflammatory processes that lead to periodontal disease (3, 28, 29).
Dental plaque isolated from healthy tissues demonstrates significant ureolytic activity (60), but the organisms that are responsible for this activity and the extent to which particular species contribute to total plaque ureolysis have not been identified (26, 61). Strains of Actinomyces naeslundii genospecies 1 (33) are gram-positive, facultatively anaerobic bacteria, characterized richly in G+C DNA content, and are usually urease positive upon isolation (55, 74), in contrast to Actinomyces viscosus (A. naeslundii genospecies 2), which is generally urease negative. Strains of A. naeslundii are of special interest because they have found almost uniquely in the mouth, they are early colonizers of the oral cavity (24, 64), and they comprise significant portions of both supragingival and subgingival dental plaque (5, 73). A. naeslundii has been implicated in the pathogenesis of root caries (57, 69) and periodontal diseases (27, 63), although these associations have never been unequivocally established (6). The organism does not appear to be involved in the development of coronal caries (73), and in fact it is most often isolated from sites with low cariogenic activity.

Our working hypothesis is that the ability of A. naeslundii to colonize the oral cavity before the emergence of acidiogenic organisms and to generate ammonia from urea in both supragingival and subgingival plaque may have a significant impact on the ecological balance in oral biofilms. To begin to understand the role of ureolysis by A. naeslundii in the physiology of oral organisms and in oral ecology and disease development, we isolated and characterized the urease gene cluster from this organism and constructed a urease-deficient, otherwise isogenic mutant strain. The isogenic mutant was compared to the wild-type organism in a number of in vitro experiments to determine the physiologic significance of urease in A. naeslundii.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and reagents. A. naeslundii WVU45 (ATCC 12104) (14) and ANUC1 (this study) were grown in either brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Mich.) or Todd-Hewitt broth (Difco) and antibiotics. All chemical reagents and antibiotics were obtained from Sigma Chemical Co. (St. Louis, Mo.).

DNA manipulations. Chromosomal DNA from Actinomyces was isolated as described by Donkerloo et al. (20). Plasmid DNA was extracted from E. coli by the rapid-boiling method (56). DNA to be used for subcloning or nucleotide sequence analysis was extracted from E. coli by using the QIA Prep Spin Plasmid Kit (Qiagen, Inc., Chatsworth, Calif.) or isolated by the method of Birnboim and Doly (2) and was further purified by centrifugation to equilibrium in cesium chloride-ethidium bromide (56). DNA was extracted from recombinant λ phages by the method of Chisholm (13). Restriction and DNA-modifying enzymes were obtained from Life Technologies Inc. (LTI) (Bethesda, Md.), or from U.S. Biochemicals (Cleveland, Ohio).

For the amplification of internal sequences of ureC from A. naeslundii by PCR, 0.5 µg of chromosomal DNA was mixed with 100 pmol of each primer (Urea-1 [5‘-AARATHCYGAYGAYTGGG-3‘] and Urea-3 [5‘-GCCGRRTDAT NGTRITAYT-3‘]) (18 to 22 bases) complementary to the deduced sequences of the A. naeslundii urease locus (obtained from LTI) and the reverse sequencing primer (Promega). Sequencing reaction products were labeled with α-35S-dATP (New England Nuclear). Genetic transfer of exogenous DNA into A. naeslundii was performed as described by Yeung and Kozelsky (79) by using a Gene Pulser (Bio-Rad Laboratories, Richmond, Calif.) connected to a pulse controller and the QIAprep Spin Miniprep Kit (Qiagen). Primer extension analysis. Total RNA was extracted from A. naeslundii WVU45 growing exponentially in BHI broth with a protocol described by Yeung (76). Primer extension was performed with the 20-mer 5‘-CGCGGACAGCAATTGATAG-3‘. The protocol used was the one of McKnight and Kingsbury (41), as described by Ausubel et al. (1) with the following modifications: primer annealing was carried out in a buffer containing 1.5 M KCl, 0.1 M Tris (pH 8.0), and 10 mM EDTA at 37°C, and reverse transcription was performed at 42°C. Terrier to measure enzyme assays. Urease activity in recombinant strains was detected by streaking onto Bacto agar base plates supplemented with 1.5% Bacto Todd-Hewitt broth (Difco) and antibiotics.

RESULTS

Isolation of the urease gene cluster from A. naeslundii WVU45. An approximately 0.6-kbp product was amplified from the A. naeslundii WVU45 genome by PCR with primers Urea-1 and Urea-3. These primers were designed based on conserved amino acid sequences of the α subunits of ureases from a number of organisms (12). The product was cloned into the PCRII vector (Invitrogen) and subjected to nucleotide sequence analysis, which revealed high levels of similarity and identity at the deduced amino acid sequence level to known ureases. Southern blot hybridization

Acid-killing experiments. In order to determine whether ureolysis could protect A. naeslundii against killing in acidic environments, the wild-type (WVU45) and mutant (ANUC1) strains were grown overnight in ADM containing 0.05% Casamino Acids. The cells were washed once in 10 mM sodium phosphate buffer (pH 7.0) and incubated at 37°C in a reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0) and incubated at 37°C in a reaction mixture containing 50 mM potassium phosphate buffer (pH 6.0) and 50 mM urea. The amount of ammonia released was quantitated with the Sigma Ammonia Color Reagent with ammonium sulfate as the standard. Urease specific activity was expressed as micromoles of ammonia produced per minute per milligram of cells (dry weight). Rapid screening for urease activity in recombinant Actinomyces strains was performed by streaking onto Bacto agar base plates supplemented with 1.5% Bacto Todd-Hewitt broth (Difco) and antibiotics.

To measure urease activity in recombinant Actinomyces strains was performed by streaking onto Bacto agar base plates supplemented with 1.5% Bacto Todd-Hewitt broth (Difco) and antibiotics.
of the PCR product, as well as of an internal fragment of the type 2 fimbrial subunit gene of *A. naeslundii* WVU45. The arrangement of the genes (top), their locations on the phage clones λLM1 and λLM9 (bottom), and the predicted molecular masses (in kilodaltons) of the corresponding proteins (numbers below the boxes) are shown. The internal fragment that was originally amplified from the chromosome by PCR and used to identify the clones is shown as a shaded box. The relative locations of the final fragment that was originally amplified from the chromosome by PCR and used to identify the clones is shown as a shaded box. The relative locations of the primer PE1, UreA-1, and UreA-3 (see text), as well as a putative proximal promoter (P), are also indicated.

The 500-bp region between the end of this ORF and the beginning of *ureA* was characterized by a large number of sequences with the potential to form strong stem-loop structures, and it contained two additional putative ORFs transcribed in the same orientation as the urease genes. The deduced amino acid sequences of these two ORFs are 40 and 58 amino acids, respectively, and they do not have homology with known proteins.

The seven ORFs encompassed a region of 5.1 kbp. The G+C content of this DNA averaged 68%, which is typical in the genus *Actinomyces*. A sequence with characteristics of a Rho-independent terminator immediately 3′ to the stop codon for the *ureD* gene was identified by use of computer algorithms. By the same method, no potential terminators appeared to be present within the intergenic regions of the urease gene cluster.

The UreA to -G proteins from *A. naeslundii* appeared to share many of the characteristics common to known bacterial urease proteins. Their calculated molecular masses (Fig. 1) corresponded well to those of the proteins from other bacteria. Amino acid residues in the structural subunits which have been shown to have functional significance in other bacterial ureases were conserved in relative position in the *A. naeslundii* urease.

Specifically, histidine residues His-134, His-136, and His-246 of the α subunit of the *K. aerogenes* urease have been shown by site-directed mutagenesis to be involved in nickel binding (53). Histidines were present at positions 137, 139, and 249 of the α subunit of *A. naeslundii* urease. His-219 and His-320 of the *K. aerogenes* α subunit are required for substrate binding and catalysis, respectively (53), and histidine residues were present at positions 222 and 315 of the *A. naeslundii* urease α-subunit. Additional amino acid residues with defined functional significance in the urease enzyme subunits include His-39 and -41 of the β subunit and His-97 of the γ subunit (53), and those were also conserved in the appropriate positions in *A. naeslundii* UreB and UreA, respectively. An amino acid sequence (MVC HHLN) which deviated by only one residue from the consensus for urease active sites (MVC HHLNL) (40, 71) was identified at amino acid positions 320 to 326 of the *A. naeslundii* UreC. The conserved ATP- and GTP-binding motif found in other UreG proteins (67) was also identified by the Genetics Computer Group MOTIFS program in *A. naeslundii* UreG (amino acid positions 32 to 39). The deduced amino acid sequence for the *A. naeslundii* ureE gene lacks a polyhistidine tail, which is thought to be involved in nickel binding by UreE of *K. aerogenes* (36, 66).

**Mapping of the transcription initiation site by primer extension.** Primer extension analysis was performed with a primer (PE1) complementary to the nucleotide sequence between positions 31 and 50 of the *ureA* coding sequence. A single transcription initiation site was identified, corresponding to a cytosine residue located at 66 bases 5′ to the start codon for *ureA* (Fig. 2). At position −4, relative to this transcription initiation site, we identified a hexanamer (−TATAAG−) with homology to the *E. coli* σ20 promoter −10 consensus sequence (TATAAT), while 19 bases 5′ to this region, a sequence (−TTCACG−) with significant homology to the *E. coli* σ20 promoter −35 consensus sequence (TTGACA) is present. The actual −10 region of the *A. naeslundii* urease promoter (−TTGCCC−) resembles the −12 consensus sequence of the *E. coli* σ20 promoter (TTGC), but no homology to this class of promoters was evident in the respective −24 region. A notable characteristic of the *A. naeslundii* urease promoter region was the unusually high frequency of adenine and thymine phosphonucleotide residues (about 45% within the −100 region).
Construction of a urease-deficient *A. naeslundii* strain. The strategy that was followed for the generation of an otherwise isogenic urease-deficient strain is shown in Fig. 3A. A 1.8-kbp *Bam*HI fragment (the second *Bam*HI site was vector derived) containing the 3′ region of *ureA*, *ureB*, and the 5′ region of *ureC* was cloned into the *E. coli* vector pUC19. A 1.3-kbp *XhoI*-DraI fragment from the broad-host-range plasmid vector pJRD215 (19), containing a kanamycin resistance gene that can be expressed in *Actinomyces* spp. (77, 79), was blunt ended and cloned into a unique *SfiI* site within the *ureC* gene. The resulting integration plasmid, pUCM1, was used to transform *A. naeslundii* WVVU45 via electroporation (79). Transformants were selected on BHI plates containing kanamycin at a concentration of 50 μg/ml. A total of 66 kanamycin-resistant *A. naeslundii* strains were obtained, all of which had a urease-negative phenotype as determined by screening on urea agar supplemented with TH and kanamycin (described in Materials and Methods). This was the predicted phenotype for mutations occurring via either single or double recombination events.

Six of the transformants were randomly selected for further characterization by Southern blot analysis. Chromosomal DNAs from these strains were extracted, digested with *Bam*HI, and run on an agarose gel. The gel was then simultaneously transferred onto two nitrocellulose membranes, one of which was probed with a urease-specific probe, a 1.1-kbp *Bam*HI-*SfiI* fragment of pUCM1, and the other of which was probed with the kanamycin resistance gene from pJRD215. Results for a single urease-negative strain, ANUC1, are presented here (Fig. 3B). The hybridization profile of this strain was consistent with insertion of the kanamycin resistance marker having occurred via a double-crossover recombination event. The same was true for one more of the six transformants that were subjected to Southern blot analysis, whereas the remaining four (66.7%) appeared to have occurred via single-crossover recombination (data not shown).

The kanamycin resistance gene was stably maintained in the chromosome of ANUC1 after passaging of the organism every day for 2 weeks in antibiotic-free medium (BHI broth). Also, the mutant strain exhibited a doubling time of approximately

![Image](http://iai.asm.org/pani/fig_2.jpg)

**FIG. 2.** Mapping of the transcription initiation site by primer extension. Lane PE1, primer extension product obtained with the primer PE1 (see text). The sequencing reactions in lanes A, C, G, and T were obtained with the same primer. Note that the sequencing reactions have been mirror-imaged in order to represent the sequence of the sense DNA strand. The nucleotide sequence of the −50 region relative to the mapped transcription initiation site is presented at the bottom. Two sequences with high homology to canonical −10 and −35 promoter sequences are underlined by dashed lines.

![Image](http://iai.asm.org/pani/fig_3.jpg)

**FIG. 3.** (A) Schematic representation of the construction of the isogenic mutant, ANUC1. (B) Southern blot analysis of genomic DNA from the wild-type (WVVU45) and urease-defective (ANUC1) *A. naeslundii* strains, probed with either a urease-specific probe or the Km' gene. The locations of the probes on the chromosomes of the two strains are indicated in panel A.
one, and glutamate. The basic solution was then diluted two-, four-, and eightfold, so that the amount of Casamino Acids was reduced to 0.1, 0.05, or 0.025%. The amount of glucose was maintained the same (0.5%) in all four dilutions of the medium. Cultures of \textit{A. naeslundii} in complete ADM reached final optical densities at 600 nm (\textit{OD},600 of 1.2. As the amount of Casamino Acids was reduced, the growth became more and more restricted, so that the final \textit{OD},600 of the cultures in ADM containing 0.1% Casamino Acids was around 0.8, that in 0.05% Casamino Acids was 0.5, and the cultures in ADM containing 0.025% Casamino Acids never grew beyond an \textit{OD},600 of 0.3 (Fig. 5). Supplementation with 25 mM NH₄Cl or 25 mM urea enhanced the growth of \textit{A. naeslundii} in all dilutions of the medium. Increasing the concentration of ammonia to 50 mM, in order to account for the differences between the number of molecules of ammonia in urea and NH₄Cl, did not increase the yield, as defined by the final OD, of the corresponding cultures (data not shown). The cultures that were supplemented with urea consistently reached higher final \textit{OD},600 values than those supplemented with NH₄Cl. However, as would be predicted, cultures grown in urea had substantially higher pH values than those grown in ammonia (Fig. 5).

To determine whether the increased final ODs of the cultures growing in urea compared to those growing in NH₄Cl were related to pH differences, growth curves in medium that had been buffered by the addition of 35 mM potassium phosphate were determined (Fig. 6). These and the following experiments were performed in ADM containing 0.05% Casamino Acids, because the differences in yields on the various nitrogen sources were most evident at this dilution and an acceptable growth could still be achieved even in the absence of nitrogen supplementation. Buffering alone did not enhance the growth of \textit{A. naeslundii} in ADM containing 0.05% Casamino Acids, compared to that in the unbuffered medium, when no additional nitrogen source was provided. Cultures that were supplemented with 25 mM urea grew equally well regardless of whether the medium was buffered. When the medium was supplemented with either 25 mM NH₄Cl or 0.2% Casamino Acids (as in the complete medium), the buffered cultures achieved \textit{OD},600 values higher than those of the unbuffered ones and comparable to those of the cultures that were supplemented with urea. Thus, it appeared that wild-type \textit{A. naeslundii} could grow almost equally well in the presence of urea, ammonia, or Casamino Acids as a nitrogen source, when the pH was not a restricting factor.

The growth of the isogenic mutant in buffered ADM containing only 0.05% Casamino Acids or supplemented with 25 mM NH₄Cl or 0.2% Casamino Acids was comparable to the growth of wild-type \textit{A. naeslundii} under the same conditions (Fig. 7). In contrast to the wild-type organism, strain ANUC1 was not able to use urea for growth in this nitrogen-limited medium, which indicated that utilization of urea by \textit{A. naeslundii} as a source of nitrogen occurs exclusively via a urease-dependent pathway.

**DISCUSSION**

Human dental plaque demonstrates high levels of ureolytic activity (60), which has been implicated in plaque pH homeostasis and plaque ecology and in the development of dental caries, calculus, and periodontal disease. Yet, the organisms that are primarily responsible for this activity have not been unequivocally identified, and molecular aspects of ureolysis in dental plaque remain largely unexplored. Of the dental plaque organisms that demonstrate ureolytic activity on isolation, \textit{Actinomyces} strains are found in significant numbers in both
supragingival and subgingival plaque and therefore have the potential to be important contributors to total plaque ureolysis (74).

Most bacterial urease operons are generally similar in their organization, being comprised of structural genes, genes involved in the incorporation of nickel into the apoenzyme, and in some cases genes involved in nickel uptake or regulation of urease expression. Differences between species usually occur in the arrangement of the genes and the spacing between them (16, 46). The gene order in the urease gene cluster of *A. naeslundii* was more similar to those of *S. salivarius* 57'I (11) and *Bacillus* species strain TB-90 (37), in that *ureD* was located in the 3' end of the cluster, rather than the 5' end as in some other organisms. Some urease clusters contain additional urease-related genes, such as the *Bacillus ureH* gene, which is thought to be involved in nickel transport (37), or the *H. pylori* and *S. salivarius ureI* genes, which have a function that is yet to be established (11, 18). No ORFs with homology to any of those genes were identified in the regions immediately 5' to *ureA* or 3' to *ureD* of *A. naeslundii*.

The 1-kbp region 5' to the coding sequence of *ureA* contains three putative ORFs transcribed in the same direction as the urease genes. The most distant of these ORFs (ORF L31) is obviously not related to known urease genes, since it is highly homologous to genes encoding 50S ribosomal proteins (L31). It seems unlikely that the remaining two ORFs are translated in vivo, because of their small size and the lack of homology of their deduced amino acid sequences to known proteins. It appears, therefore, that no additional ORFs that could be cotranscribed with the urease genes exist within the 500-bp region between ORF L31 and the urease cluster. The transcriptional initiation site for the urease genes was mapped in this region, at 66 bases 5' to the start codon for *ureA*. Given the observations that the urease genes are tightly spaced and that there is a lack of potential Rho-independent terminators in the intergenic regions, it is possible that these genes constitute an operon, similar to the case for numerous other urease gene clusters. We have made several attempts, using multiple RNA isolation procedures and growth conditions, to identify transcripts with *ure* gene probes. These efforts have been impeded by the comparatively low abundance and apparent short half-life of the corresponding transcripts and have been further complicated by substantial degradation of the RNA, which is
neutral pH values than those found in the cultures that utilized the cultures with urea were invariably associated with more ammonia or a mixture of amino acids. The higher ODs of nitrogen source always reached higher ODs than those growing mentally acidification within a comparatively narrow range of 3.0, yet it considerably increased its survival at pH 4.0. Although the exact levels of protection against environmental acidification provided by ureolysis were difficult to determine due to problems reported in Results, our data suggest that urease may confer to *A. naeslundii* protection from acid-induced damage during growth at clinically relevant pH values, i.e., in a range between 4.0 and 7.5.

The ability of *A. naeslundii* to utilize urea as a nitrogen source and as a means of protection against environmental acidification could constitute an important ecological determinant in dental plaque, especially when fermentable carbohydrates are present in excess. A carbohydrate-rich diet is known to promote the overgrowth of acidogenic mutants streptococci and lactobacilli in dental plaque, at the expense of less acidogenic organisms, such as *Streptococcus sanguis* and the *Actinomyces* spp. (4, 8, 10). The potential of plaque bacteria to withstand this major ecological pressure depends on their abilities to compete for nutrients and to survive in the acidic environment generated by the increased production of organic acids from glycolysis. The utilization of urea could confer to *A. naeslundii* two significant competitive advantages under these conditions. First, it would provide efficient access to a highly abundant nitrogen source, since urea is continuously supplied in saliva and gingival crevicular fluid at concentrations that range from 3 to 10 mM (28). This source is not available to the nonureolytic mutants streptococci, which have to rely heavily on the more limited amino acid and oligopeptide pools for protein synthesis. Second, as our data indicate, the production of ammonia from urea could neutralize organic acids produced during this period of increased glycolytic activity in plaque and render the environmental pH more favorable for itself and other less-acid-tolerant organisms (15). Since the balance between the acidogenic and the less acidic organisms is one of the most important determinants of caries susceptibility (72), ureolysis by *A. naeslundii* could reduce the cariogenic potential of plaque by preventing the shift to a very acidogenic flora. The effects of the *A. naeslundii* urease in oral ecology could be even more significant during the early stages of colonization of the oral tissues, since *A. naeslundii* is one of the pioneer organisms in the oral cavity and is an early colonizer of the tooth surface.

In addition to it being one of the very first organisms to colonize the human oral cavity, one of the most important properties of *A. naeslundii* is probably its ability to successfully thrive both above and below the gingival margin. Supragingival and subgingival dental plaque constitute two distinct ecological niches, and the generation of ammonia from urea by *A. naeslundii* could have a totally different clinical impact in each location. This could possibly explain the controversial pathogenic profile of this organism. In supragingival plaque, ureolysis by *A. naeslundii* could modulate glycolytic acidification and inhibit dental caries, while the production of ammonia and elevation of pH by the same organism in subgingival plaque could promote calculus formation and periodontal inflammation. Our urease-defective strain, if able to be established in an appropriate animal model, could be very useful in helping us to understand the potential contribution of the *A. naeslundii*
urease in oral ecology, dental caries prevention, and possibly the formation of calculus and the pathogenesis of periodontal diseases.

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