Evidence for recombination between a sialidase (nanH) of Actinomyces naeslundii and Actinomyces oris, previously named ‘Actinomyces naeslundii genospecies 1 and 2’

Thuy Do, Uta Henssge, Steven C. Gilbert, Douglas Clark & David Beighton

King’s College, School of Medicine and Dentistry, London, UK

OnlineOpen: This article is available free online at www.blackwell-synergy.com

Correspondence: David Beighton,
Department of Microbiology, King’s College
London, Dental Institute, Floor 17, Guys
Tower, London Bridge, London SE1 9RT, UK.
Tel.: +44 20 7188 7465; fax: +44 20 7188 7466; e-mail: david.beighton@kcl.ac.uk

Received 20 June 2008; accepted 31 July 2008.
First published online 24 September 2008.
DOI:10.1111/j.1574-6968.2008.01336.x
Editor: Robert Burne

Keywords
Actinomyces; sialidase; dental plaque.

Abstract
Actinomyces spp., predominant members of human oral biofilms, may use extracellular sialidase to promote adhesion, deglycosylate immunoglobulins and liberation of nutrients. Partial nanH gene sequences (1077 bp) from Actinomyces oris (n = 74), Actinomyces naeslundii (n = 30), Actinomyces viscosus (n = 1) and Actinomyces johnsonii (n = 2) which included the active-site region and the bacterial neuraminidase repeats (BNRs) were compared. The sequences were aligned and each species formed a distinct cluster with five isolates having intermediate positions. These five isolates (two A. oris and three A. naeslundii) exhibited interspecies recombination. The nonsynonymous/synonymous ratio was < 1 for both A. oris and A. naeslundii indicating that nanH in both species is under stabilizing selective pressure; nonsynonymous mutations are not selected. However, for A. oris significant negative values in tests for neutral selection suggested the rate of mutation in A. oris was greater than in A. naeslundii but with selection against nonsynonymous mutations. This was supported by the observation that the frequency of polymorphic sites in A. oris, which were monomorphic in A. naeslundii was significantly greater than the frequency of polymorphic sites in A. naeslundii which were monomorphic in A. oris (χ² = 7.011; P = 0.00081). The higher proportions of A. oris in the oral biofilm might be explained by the higher mutation rate facilitating an increased ability to respond successfully to environmental stress.

Introduction
Sialidase activity is produced by many commensal oral bacteria including Actinomyces spp. (Moncla & Braham, 1989; Beighton & Whiley, 1990; Beighton et al., 1991; Braham & Moncla, 1992). The activity produced by Actinomyces spp. desialylates IgA by the removal of the terminal sialic acid residues rendering the molecule more susceptible to proteolysis (Reinholdt et al., 1990; Frandsen, 1994). Sialidase activity also appears important in the nutrition of the oral biofilm because sialidase activity increases in the absence of host diet, and withdrawal of the normal diet increases the proportions of sialidase-producing bacteria (Smith & Beighton, 1986; Lucas et al., 1997; Sheehy et al., 2000). Sialic acid is utilized by various oral streptococci, Actinomyces naeslundii and by different mixed populations of subgingival plaque bacteria (ter Steeg et al., 1987; Frandsen, 1994; Byers et al., 1996, 1999; Homer et al., 1996). Sialidase also exposes galactose residues of O- and N-linked glycans which mediate the adherence of human A. naeslundii strains described previously as genospecies 1 and 2 to human glycans including those of the salivary pellicle (Costello et al., 1979; Gibbons et al., 1990). The complete genome of ‘A. naeslundii’ MG1 (http://cmr.jcvi.org/tigr-scripts/CMR/CmrHomePage.cgi) contains ORFs for two sialidase genes (ANA2709, nanH, a sialidase and ANA1493 an exosialidase). The sequence of the nanH gene (ANA2709) of MG1 has > 97% similarity with the sequences of the two previously reported Actinomyces sialidase gene sequences (Henningsen et al., 1991; Yeung, 1993) while the ANA1493 exhibits < 5% sequence similarity with the nanH genes.
Two of the strains from which the nanH was sequenced were described as *Actinomyces viscosus* but such human strains were subsequently described as *A. naeslundii* genospecies 2 (Johnson *et al.*, 1990). However, on the basis of a phylogenetic analysis of partial gene sequences of housekeeping genes, we (Henssge *et al.*, in press) have reported that strains previously identified as *A. naeslundii* genospecies 2, including the sequenced strain MG1, should be reclassified as *Actinomyces oris*. The species *A. naeslundii* has been amended to include only strains previously identified as *A. naeslundii* genospecies 1. Therefore, we have nanH sequence data on three *A. oris* strains but no information of the nanH genes of *A. naeslundii*, *Actinomyces johnsonii* (previously *A. naeslundii* genospecies WVA 963) or animal strains of *A. viscosus*.

In this report, we have compared the nucleotide and amino acid sequences of the region of the nanH gene containing the active site (Crennell *et al.*, 1993) and the five Asp boxes or bacterial neuraminidase repeats (BNRs, Henningsen *et al.*, 1991) of these *Actinomyces* species. In the mouth *A. naeslundii* and *A. oris* occupy the same sites but *A. oris* is the predominant species (Bowden *et al.*, 1999) while *A. johnsonii* was isolated from the gingival crevice (Johnson *et al.*, 1990). We present a phylogenetic analysis of a partial sequence of nanH in these four species and present evidence of interspecies recombination between nanH genes.

### Materials and methods

#### Bacterial strains

The isolates have all been reported and identified in a previous taxonomic study (Henssge *et al.*, in press) describing the new species *A. oris* (previously *A. naeslundii* genospecies 2) and *A. johnsonii* (previously *A. naeslundii* genospecies WVA 963) and reporting an emended description of *A. naeslundii* (previously *A. naeslundii* genospecies 1). The strains included in this study were 30 *A. naeslundii* (CCUG 33521, CCUG 33522, CCUG 35199, CCUG 33523, ATCC 12104, CCUG 34725, CCUG 35334 and CCUG 37599 and 22 human clinical and oral isolates); 71 *A. oris* (P2G, P5K, P6K, P7K, P8K, P9K, Pn4D, Pn5D, CCUG 33915, CCUG 33919, CCUG 33920, CCUG 33914, CCUG 34285, CCUG 34286 and ATCC 27044 and 56 human clinical and oral isolates); two *A. johnsonii* (CCUG 33932 and CCUG 34287) isolates and one *A. viscosus* (NCTC 10951). The oral and clinical isolates were given study numbers from 1 to 94. All isolates were cultured anaerobically at 37 °C on Fastidious Anaerobe Agar (LabM) supplemented with 5% v/v defibrinated horse blood and stored at −80 °C in brain–heart infusion (Oxoid) containing 50% glycerol.

#### PCR conditions and nanH sequencing

DNA was extracted from cells as described previously (Henssge *et al.*, in press). Owing to the length of the gene fragment which was likely to contain the sites of interest, two pairs of primers were used to obtain a 1077-bp fragment of the nanH gene in all species: Sial-F1 5′-ACACGATCAGCAAGCCA-GA-3′ and Sial-R1 5′-CGACCTTGTTCTCATCACA-3′ and Sial-F2 5′-AACCACTCGTCCA-3′ and Sial-R2 5′-GAGCCGATTCATCGTGA-3′. The PCR products were performed using ReddyMix (Abgene, Epsom, Surrey, UK) and the PCR conditions for each pair of primers were an initial denaturation for 10 min at 94 °C followed by 30 cycles of 94 °C for 30 s, 49 °C for 30 s and 72 °C for 90 s. A final extension was carried out for 5 min at 72 °C. The PCR products were visualized on a 1% agarose gel stained with Gel Red (Biotium Inc.). The amplicons were cleaned using a 50:50 mixture of 40% polyethylene glycol and 3 M NaCl, washed twice with 70% ethanol and rehydrated in sterile water. The same primers were used for the sequencing reactions and all amplicons were sequenced in both directions using the BigDye Terminator Sequencing kit (Applied Biosystems) and reaction products were run on a 3730xl sequencer (Applied Biosystems).

#### nanH sequence analysis

The DNA sequences were aligned using BIOEDIT (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The phylogenetic relationships between partial nanH nucleotide sequences of the reference strains, the human oral and clinical isolates and the two ′A. viscosus′ nanH sequences in GenBank (L06898 and X62276) and the nanH of the sequenced strain MG1 (ANA2709) were analysed using MEGA 4 (Tamura *et al.*, 2007). Distances were calculated using the Kimura two-parameter model and for clustering the neighbour-joining method of Saitou & Nei (1987) using bootstrap values based on 500 replicates was used. The amino acid sequences were clustered using CLUSTALW2 (http://www.ebi.ac.uk/).

DNSP (Rozas *et al.*, 2003) was used to investigate the nanH sequences of *A. naeslundii* and *A. oris*. The G+C content of the partial genes sequences, the number of discrete sequences, the number of polymorphic sites, average nonsynonymous/synonymous ratios (dN/dS) were calculated and nucleotide diversity was estimated by determining (π) (nucleotide diversity) and (θ) (the total number of mutations). To test for neutral molecular evolution three tests were used; Tajima's *D*, based on the differences between the number of segregating sites and the average number of nucleotide differences, Fu and Li's *D*, based on the differences between the number of singletons (mutations appearing only once among the sequences) and the total number of mutations and Fu and Li's *F*, based on the
differences between the number of singletons, and the average number of nucleotide differences between pairs of sequences. The extent of the DNA sequence polymorphism between *A. naeslundii* and *A. oris* was calculated and compared using a *χ*² test.

Split decomposition trees were constructed with 1000 bootstrap replicates based on parsimony splits as implemented in the SPLITSTREE 4.0 (Hudson & Bryant, 2006) and the statistic phi was calculated. To identify strains with evidence of recombination the Recombination Detection Package (Martin *et al*., 2005) was used with the default settings.

**Identification of BNR sequences and active site residues**

The BNRs were identified using the InterProScan Sequence Search tool (http://www.ebi.ac.uk/Tools/InterProScan/). The amino acid polymorphisms in the BNRs were determined by visual inspection. The partial *nanH* amino acid sequences of the four type strains were aligned with the sequence of a *Salmonella typhimurium* sialidase (M55342) using Parallel Protein Information Analysis System (http://www.cbrc.jp/papia/papia.html) and the 12 putative active site residues of the *S. typhimurium* sialidase (Crennell *et al*., 1993) were identified.

**Detection of *nanH* transcripts**

Each type strain was grown on FAA supplemented with 5% (v/v) defibrinated horse blood and total RNA was extracted using the UltraClean Microbial RNA Isolation Kit (MO BIO Laboratories Inc.). Reverse transcription was performed using the UltraClean Microbial RNA Isolation Kit (MO BIO Laboratories Inc.). Reverse transcription was performed using the Omniscript RT kit (Qiagen Ltd) with primers, Sial-F1 and Sial-R1, for *nanH* and AtpA-F (CCCTGGAG TAGACCCCAT) and AtpA-R (GGCCAGGGTGATCTTGAG), to amplify the housekeeping gene, *atpA* (ATP synthase F1, α subunit, ANA_0169). The thermal cycling was as follows: cDNA synthesis at 37 °C for 30 min, 94 °C for 1 min, followed by amplification with 40 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and then the final extension at 72 °C for 5 min. The products were run on a 1% agarose gel, with Gel Red incorporated, for 10 min at 100 V. The identity of the products was confirmed by sequencing as described above.

**Analysis**

The amino acid sequences of the BNRs of the *A. naeslundii* type strains 25, 51 and CCUG 34725 and of the *A. oris* strains 60 and 61 (each shown with the *nanH* sequence accession number) did not cluster with the majority of strains of the same species and were identified as strains with significant evidence of interspecies recombination. Scale bar = 0.01 substitutions per site.

![Fig. 1. Neighbour-joining tree showing relationships between type and reference strains of Actinomyces naeslundii, Actinomyces oris, Actinomyces johnsonii and Actinomyces viscosus and oral and clinical isolates determined by partial *nanH* gene sequence analysis. Actinomyces naeslundii strains 25, 51 and CCUG 34725 and A. oris strains 60 and 61 (each shown with the *nanH* sequence accession number) did not cluster with the majority of strains of the same species and were identified as strains with significant evidence of interspecies recombination. Scale bar = 0.01 substitutions per site.](https://academic.oup.com/femsle/article-abstract/288/2/156/528385)

analysis of the derived amino acid sequences (data not shown) with the same isolates being found on the periphery of the clusters composed of the majority of the *A. oris* and *A. naeslundii* isolates and clearly distinct from the *A. johnsonii* and *A. viscosus* sequences.

The dN/dS values for *A. oris* and *A. naeslundii* were 0.1466 and 0.2134, respectively. Tajima’s D, Fu and Li’s *D* and *F* were all significantly negative for *A. oris* but did not achieve significance for *A. naeslundii* (Table 1). The test for DNA divergence between populations implemented in DNASP indicated that the number of polymorphic sites in *A. naeslundii* and monomorphic in *A. oris* was 59 but the number of polymorphic sites in *A. oris* and monomorphic in *A. naeslundii* was 287 (*χ*² = 7.011; *P* = 0.00081).

Analysis of the amino acid sequences of the BNRs of the four species is shown in supporting Table S1. There was overall great consistency in the pattern SXDXGXTW within each of the BNRs for all of the species with only single exceptions in the first for BNRs. In the fifth BNR the pattern...
was SXDXGXS in all of the isolates except for one A. johnsonii isolate which had the sequence SCGNGASW.

The alignment of the amino acid sequences inferred from the partial nanH sequences from the four species with that of S. typhimurium LT2 demonstrated that the active site identified in the S. typhimurium sialidase was recognized in each of the Actinomyces sequences. Nine of the 12 amino acids in the S. typhimurium active site were identical in all the Actinomyces sequences (supporting Fig. S1). The exceptions among the sequences were the met-99 and trp-121 in S. typhimurium which were both replaced by serine and leu-175 which was replaced by phenylalanine.

To test for the statistical evidence of recombination in the first instance the Splitstrees method was used and the phi test provided evidence of significant recombination when all strains, except the A. viscosus and A. johnsonii strains, were included in the analysis (\(P = \times 10^{-20}\)). Consideration of A. oris or A. naeslundii strains alone yielded phi values with \(P = 3.43 \times 10^{-6}\) and \(P = 1.37 \times 10^{-3}\), respectively, indicating statistically significant evidence of recombination in each of the species. To identify the strains with evidence of recombination we analysed the data of these two species together using the seven programs within the RDP suite and found that only A. naeslundii strains 25, 51 and CCUG 34725 and A. oris strains 60 and 61 gave significant evidence of recombination. The recombination events are summarized in Fig. 2. In the A. naeslundii strains 25, 51 and CCUG 34725 and A. oris strain 60 the recombination event extended beyond the available partial sequences.

The type strains of each of the four species examined expressed the nanH gene when grown on a complex medium containing defibrinated horse blood and 1% (w/v) glucose (Fig. 3) and sequence analysis confirmed that the individual products were derived from the nanH genes of the appropriate species.

![Fig. 3](image-url) Reverse transcriptase (RT)-PCR assays showing expression of nanH and the housekeeping gene atpA in the type strains of Actinomyces oris (CCUG 34288; EU805602), Actinomyces johnsonii (CCUG 34287; EU805600), Actinomyces naeslundii (CCUG 2238; EU805601) and Actinomyces viscosus (NCTC 10951; EU805603). Negative controls, omitting the RT in the reaction mix, yielded no amplicons from any strain.

![Fig. 2](image-url) Recombination events found in nanH of Actinomyces oris strains 60 (EU805671) and 61 (EU805672) and Actinomyces naeslundii strains 51 (EU805620), 25 (EU805612) and CCUG 34725 (EU805609). Solid line indicates portion of sequence derived from nanH of A. naeslundii and broken line indicates portion of sequence derived from nanH of A. oris. Insertion in strain 25 between 365 and 1038, in strain 51 between 629 and 1038, in strain 60 between 1 and 477, in strain 61 between 432 and 1038 and between 1 and 655 in CCUG 34725. Breakpoints determined using RDP suite of programs with significant evidence (\(P < 0.001\)) for recombination obtained with \(\geq 5\) recombination tests in all cases.

### Table 1. Genetic variation of partial nanH sequences of type and reference strains and clinical isolates of Actinomyces oris and Actinomyces naeslundii

|                      | A. naeslundii (n = 30) | A. oris (n = 74) |
|----------------------|------------------------|------------------|
| G+C content          | 0.687                  | 0.693            |
| No. of segregating sites, S | 147                   | 319              |
| No. of discrete sequences | 29                    | 71               |
| dN/dS                | 0.2134                 | 0.1466           |
| \(\pi\)              | 0.0210                 | 0.0296           |
| \(\theta\)           | 0.0361                 | 0.0728           |
| Tajima’s D           | -1.6094                | -2.0658**        |
| Fu and Li’s D*       | -1.9615                | -3.6456**        |
| Fu and Li’s F*       | -1.6815                | -3.5907**        |

\(\pi\) and \(\theta\), nucleotide diversity.

All analyses were performed using DNASP version 4.5 (http://www.ub.es/dnap/index.html).

\(*P < 0.05; **P < 0.02\).
Discussion

The complete functions of sialidase in the oral cavity are certainly not fully understood but dietary restriction in macaque monkeys resulted in the increase in levels of sialidase activity in the oral biofilm suggesting a role in bacterial nutrition (Beighton & Smith, 1986). The levels of sialidase-producing bacteria also increase in the absence of host’s diet, again supporting the hypothesis that it plays a role in providing nutrient, both C and N, for microbial growth and proliferation (Beighton & Smith, 1986; Lucas et al., 1997; Sheehy et al., 2000). Bacterial sialidases, including those of the oral Actinomyces, also express galactose residues of glycoprotein glycans located on mucosal surfaces or adsorbed to the tooth as part of the enamel pellicle. The exposed galactose residues may then be used as attachment sites by members of the oral biofilm promoting dental plaque accumulation (Costello et al., 1979; Gibbons et al., 1990).

All sialidase enzymes have a highly conserved array of residues, which in the tertiary structure of the enzyme, form the active and binding sites of the molecule (Crennell et al., 1993). In addition to these residues all sialidases also have a number of BNRs structural entities for which a function cannot be definitely ascribed because Asp boxes are found in many protein families (Henningsen et al., 1991; Copley et al., 2001). In determining the sequence diversity of nanH in these Actinomyces species, we selected a portion of the gene which contained the 12 residues involved in substrate interactions with, and stabilization of, the active site and the BNRs originally described in A. viscosus, now A. johnsonii (Crennell et al., 1993). The methods used to interrogate data for the presence of recombinational events provided evidence for significant but limited recombination between the A. oris and A. naeslundii. This is the first evidence of recombination between these two species although recombination between sialidase genes of other oro-pharyngeal organisms, Streptococcus oralis and Streptococcus pneumoniae, has been reported (King et al., 2005). We found evidence of recombination in three of 30 A. naeslundii strains and in two of 74 A. oris isolates suggesting that recombination between these species is either not uncommon or these may be rare events and the recombinants may be more successful than the parent strains. However, the growth or survival benefit acquired by these recombinant strains is not known because they have persisted and proliferated, at least in the mouths of the individuals from whom they were isolated, this suggests benefit has been accrued. The survival

region have also been reported in other high G+C organisms (Sakurada et al., 1992; Jost et al., 2001).

The dN/dS values were < 1 for both A. oris and A. naeslundii indicating that nanH in both species is under stabilizing selective pressure; nonsynonymous mutations are not selected. However, for A. oris significant negative values Tajima’s D and Fu and Li’s D* and F* were calculated suggesting that, as all nucleotides of any codon are assumed to be equally mutatable, the rate of mutation in A. oris must be greater than in A. naeslundii but with selection against nonsynonymous mutations. The proposed higher rate of mutation in the nanH of A. oris was supported by the observation that the frequency of polymorphic sites in A. oris which were monomorphic in A. naeslundii was significantly greater than the frequency of polymorphic sites in A. naeslundii which were monomorphic in A. oris. Consideration of our previously published sequence data on six housekeeping genes (Hensge et al., in press) indicated that the frequency of polymorphic sites in A. oris which were monomorphic in A. naeslundii was also significantly (P < 0.05) greater in gltA, metG, pgI and rpoB, while in gyrA and atpA no significant difference was found. The reasons for the apparent greater frequency of mutations in A. oris is not known but may suggest that A. oris is subject to greater stress than A. naeslundii in the oral biofilm because environmental stress may increase the rate of mutation of organisms in biofilms (Bjedov et al., 2003; Tenaillon et al., 2004). However, given the structural requirements of a functioning sialidase, nonsynonymous mutations may not be beneficial and will, therefore, not be selected. Actinomyces oris (as A. naeslundii genospecies 2) is numerically more successful in the oral biofilm than A. naeslundii (as A. naeslundii genospecies 1) (Bowden et al., 1999) which might in part be explained by its higher mutation rate facilitating genome plasticity and an increased ability to respond successfully to environmental stress.
and growth benefits may not be due to recombinational events in $\text{n}anH$ but to other unknown events, congruent with possession of a recombinant $\text{n}anH$ gene, which might be responsible for the proliferation of these particular strains.

**Acknowledgements**

This project was supported by a grant from the Wellcome Trust (GR076381MA). The authors would like to thank Professor Nicklas Stromberg, Umeå University, Sweden, for gift of many of the Actinomyces strains used in this study; other strains were purchased from the Culture Collection, University of Göteborg, Sweden, the National Collection of Type Cultures, HPA, Colindale, England and the human clinical isolates were purchased from Dr Val Hall, Anaerobe Reference Unit, Department of Medical Microbiology and Public Health Laboratory, University Hospital of Wales, Cardiff.

**Note**

$\text{n}anH$ partial gene sequences have been deposited in GenBank with accession numbers EU805600–EU805702.

**Statement**

Re-use of this article is permitted in accordance with the Creative Commons Deed, Attribution 2.5, which does not permit commercial exploitation.

**References**

Beighton D & Smith K (1986) The modulation of exoglycosidic enzymes in the supragingival plaque of macaque monkeys. *Arch Oral Biol* **34**: 319–322.

Beighton D & Whiteley RA (1990) Sialidase activity of the “Streptococcus milleri group” and other viridans group streptococci. *J Clin Microbiol* **28**: 1431–1433.

Beighton D, Hardie JM & Whiteley RA (1991) A scheme for the identification of viridans streptococci. *J Med Microbiol* **35**: 367–372.

Bjedov I, Tenaillon O, Gérad B, Souza V, Denamur E, Radman M, Taddei F & Matic I (2003) Stress-induced mutagenesis in bacteria. *Science* **300**: 1404–1409.

Bowden GH, Nolette N, Ryding H & Cleghorn BM (1999) The diversity and distribution of the predominant ribotypes of *Actinomyces naeslundii* genospecies 1 and 2 in samples from enamel and from healthy and carious root surfaces of teeth. *J Dent Res* **78**: 1800–1809.

Braham PH & Moncla BI (1992) Rapid presumptive identification and further characterization of *Bacteroides forsythus*. *J Clin Microbiol* **30**: 649–654.

Byers HL, Homer KA & Beighton D (1996) Utilization of sialic acid by viridans streptococci. *J Dent Res* **75**: 1564–1571.

Byers HL, Tarelli E, Homer KA, Hambly H & Beighton D (1999) Growth of viridans streptococci on human serum alpha1-acid glycoprotein. *J Dent Res* **78**: 1370–1380.

Copley RR, Russell RB & Ponting CP (2001) Sialidase-like Asp-boxes: sequence-similar structures within different protein folds. *Protein Sci* **10**: 285–292.

Costello AH, Cisar JO, Kolenbrander PE & Gabriel O (1979) Neuraminidase-dependent hamagglutination of human erythrocytes by human strains of *Actinomyces viscosus* and *Actinomyces naeslundii*. *Infect Immun* **26**: 563–572.

Crennell SJ, Garman EF, Laver WG, Vinnr ER & Taylor GL (1993) Crystal structure of a bacterial sialidase (from *Salmonella typhimurium* LT2) shows the same fold as an influenza virus neuraminidase. *Proc Natl Acad Sci USA* **90**: 9852–9856.

Frandsen EV (1994) Carbohydrate depletion of immunoglobulin A1 by oral species of gram-positive rods. *Oral Microbiol Immunol* **9**: 352–358.

Gibbons RJ, Hay DI, Childs WC III & Davis G (1990) Role of cryptic receptors (cryptitopes) in bacterial adhesion to oral surfaces. *Arch Oral Biol* **35**: (suppl): 1075–114S.

Henningsen M, Roggentin P & Schauer R (1991) Cloning, sequencing and expression of the sialidase gene from *Actinomyces viscosus* DSM 45798. *Biol Chem Hoppe Seyler* **372**: 1065–1072.

Henssge U, Do T, Radford DR, Gilbert SC, Clark D & Beighton D (2008) Emended description of *Actinomyces naeslundii* and descriptions of *Actinomyces oris* sp. nov. and *Actinomyces johnsonii* sp. nov. previously identified as *Actinomyces naeslundii* genospecies 1 and 2 and WVA 963. *Int J Syst Evol Microbiol* (in press).

Homer KA, Kelley S, Hawke J, Beighton D & Grootveld MC (1996) Metabolism of glycoprotein-derived sialic acid and N-acetylglucosamine by *Streptococcus oralis*. *Microbiology* **142**: 1221–1230.

Hudson DH & Bryant D (2006) Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol* **23**: 254–267.

Johnson JL, Moore L, Kaneko B & Moore WE (1990) *Actinomyces georgiae* sp. nov., *Actinomyces gerencseriae* sp. nov., designation of two genospecies of *Actinomyces naeslundii*, and inclusion of *A. naeslundii* serotypes II and III and *Actinomyces viscosus* serotype II in *A. naeslundii* genospecies 2. *Int J Syst Bacteriol* **40**: 273–286.

Jost BH, Songer JG & Billington SJ (2001) Cloning, expression, and characterization of a neuraminidase gene from *Arcanobacterium pyogenes*. *Infect Immun* **69**: 4430–4437.

King SJ, Whatmore AM & Dawson CG (2005) *Nana*, a neuraminidase from *Streptococcus pneumoniae*, shows high levels of sequence diversity, at least in part through recombination with *Streptococcus oralis*. *J Bacteriol* **187**: 5376–5386.

Lucas VS, Beighton D, Roberts GI & Challacombe SJ (1997) Changes in the oral streptococcal flora of children undergoing allogeneic bone marrow transplantation. *J Infect* **35**: 135–141.
Martin DP, Williamson C & Posada D (2005) RDP2: recombination detection and analysis from sequence alignments. Bioinformatics 21: 260–262.

Moncla BJ & Braham P (1989) Detection of sialidase (neuraminidase) activity in Actinomyces species by using 2’-(4-methylumbelliferyl)alpha-2-N-acetylneuraminic acid in a filter paper spot test. J Clin Microbiol 27: 182–184.

Reinholdt J, Tomana M, Mortensen SB & Kilian M (1990) Molecular aspects of immunoglobulin A1 degradation by oral streptococci. Infect Immun 58: 1186–1194.

Rozas J, Sánchez-DelBarrio JC, Meseguer X & Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19: 2496–2497.

Saitou N & Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425.

Sakurada K, Ohta T & Hasegawa M (1992) Cloning, expression, and characterization of the Micromonospora viridifaciens neuraminidase gene in Streptomyces lividans. J Bacteriol 174: 6896–6903.

Sheehy EC, Beighton D & Roberts GJ (2000) The oral microbiota of children undergoing liver transplantation. Oral Microbiol Immunol 15: 203–210.

Smith K & Beighton D (1986) The effects of the availability of diet on the levels of exoglycosidases in the supragingival plaque of macaque monkeys. J Dent Res 65: 1349–1352.

Tamura K, Dudley J, Nei M & Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol and Evolution 24: 1596–1599.

Tenaillon O, Denamur E & Matic I (2004) Evolutionary significance of stress-induced mutagenesis in bacteria. Trends Microbiol 12: 264–270.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Comparison of the amino acid sequence of bacterial neuraminidase repeats [BNRs] and frequency of detection amongst A. naeslundii, A. oris, A. johnsonii and A. viscosus strains.

Fig. S1. Alignment of partial sequences of the nanH gene of the type strains of A. naeslundii (accession number EU805601), A. oris (EU805602), A. johnsonii (EU805600) and A. viscosus (EU805603) with the nanH gene sequence of S. typhimurium (M55342).

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.