

# The novel species *Streptococcus tigurinus* and its association with oral infection

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*Streptococcus tigurinus* is a novel species of viridans streptococci, shown to cause severe invasive infections such as infective endocarditis, spondylodiscitis and meningitis. *S. tigurinus* belongs to the *Streptococcus mitis* group and is most closely related to *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus pneumoniae*, *Streptococcus pseudopneumoniae* and *Streptococcus infantis*. The presence of *S. tigurinus* in the human oral cavity has been documented, including in patients with periodontal disease. This review addresses the available scientific knowledge on *S. tigurinus* and its association with closely related streptococci, and discusses its putative involvement in common oral infections. While there is as yet no strong evidence on the involvement of *S. tigurinus* with oral infections, its presence in the oral cavity and its association with endocarditis warrants special attention for a link between oral and systemic infection.

## Introduction

The human oral microbiome consists of a number of bacteria; most of them are non-pathogenic commensals or act as opportunistic pathogens.<sup>1</sup> Some oral bacteria are implicated in oral diseases such as dental caries and periodontitis, which are among the most common infections in humans.<sup>2,3</sup> Several oral bacteria have the capacity to form biofilms, which are built by complex polymicrobial mechanisms on surfaces of teeth or soft oral mucosa.<sup>4</sup> Viridans streptococci form a major part of the human oral microbiome, comprising 4 species groups: namely salivarius, anginosus, mutans and mitis group, respectively.<sup>5</sup> *Streptococcus mutans* is a key player in the development of dental caries,<sup>6</sup> and *Streptococcus anginosus* is detected in periapical odontogenic abscesses,<sup>7</sup> while the other species groups are less known as causative agents of oral diseases. Some bacterial species are rather associated with oral health than disease, e.g., *Streptococcus salivarius*,

which is commonly found on mucosal surfaces and in the saliva.<sup>8</sup> Interestingly, the filamentation capacity of *S. salivarius* is markedly increased compared to other viridans streptococci, a property which may be required for a more efficient adhesion onto continuously shedding oral mucosal surfaces.<sup>9</sup> Other more virulent bacteria potentially enter the bloodstream, which increases the risk for invasive infections, e.g., infective endocarditis.<sup>10</sup> *Streptococcus mitis*, a prominent representative of viridans streptococci, is a leading cause of infective endocarditis.<sup>11</sup>

Recently, a novel pathogen associated with severe invasive infections such as infective endocarditis was described: *Streptococcus tigurinus* belonging to viridans streptococci.<sup>12–14</sup> *S. tigurinus* initially was detected in an elderly patient with infective endocarditis.<sup>12</sup> First, a viridans streptococcal organism was isolated in multiple blood cultures of the patient. Then, the bacterial strain was analyzed by 16S rRNA gene sequencing for accurate species identification. Analysis of the aortic valve specimen of the patient by direct 16S rRNA gene broad-range PCR<sup>15</sup> revealed an identical sequence compared to that of the isolate from the blood. 16S rRNA gene sequence comparison to validated sequences in the public database showed highest sequence similarity to the strain *S. mitis* ATCC 15914 with 99.9% identity (GenBank accession number AY281076). The next best match in the database was the type strain of the species *S. mitis* ATCC 49456<sup>T</sup> (AY485601) but only with 98.6% sequence identity. Obviously, this was a novel species because of the high sequence demarcation of 1.3%. Additionally, the strain ATCC 15914, which was typed in 1977 based solely on phenotypic characteristics, must have been erroneously assigned to the species *S. mitis*.<sup>16</sup> Other authors have questioned the correct species assignment of strain ATCC 15914 on basis of analyses of the housekeeping genes *zwf* and *gki*<sup>17</sup> and the ribosomal 16S–23S intergenic spacer region.<sup>18</sup> Deeper analyses by different methods such as conventional biochemical testing, molecular analyses and DNA–DNA hybridization techniques proofed the presence of a novel species for which the name *S. tigurinus* was assigned.<sup>12</sup>

### *S. tigurinus* is a member of the *Streptococcus mitis* group

*S. tigurinus* belongs to the *S. mitis* group, which consists of different species, i.e., *Streptococcus pneumoniae*, *Streptococcus pseudopneumoniae*, *S. mitis*, *Streptococcus oralis*, *Streptococcus infantis*, *Streptococcus sanguinis*, *Streptococcus parasanguinis*, *Streptococcus cristatus*, *Streptococcus gordonii*, *Streptococcus peroris*, *Streptococcus australis*, *Streptococcus oligofermentans* and *Streptococcus sinensis*.<sup>19,20</sup> Recently, 3 novel species were assigned to the *S. mitis*

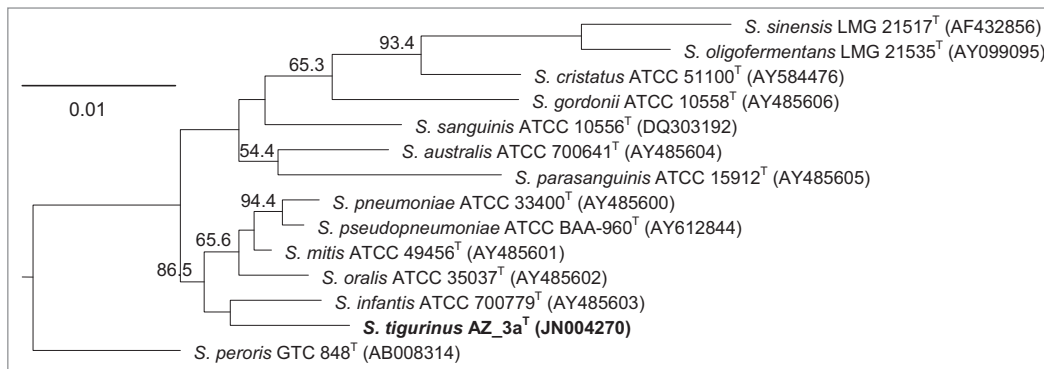
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**Figure 1.** Phylogenetic analysis of the *Streptococcus mitis* group. The neighbor-joining phylogenetic tree based on partial 16S rRNA gene sequences (>1300 bp) shows the relationships among *S. tigurinus* strain AZ\_3a<sup>T</sup> and related species within the *S. mitis* group. Bootstrap percentages (based on 1000 replications) > 50% are shown at branching points. Published sequences used were from the public GenBank database. Bar, 0.01 substitutions per nucleotide position.

group, i.e., *Streptococcus dentisani*,<sup>21</sup> *Streptococcus rubneri*<sup>22</sup> and *Streptococcus lactarius*.<sup>23</sup> *S. dentisani* was isolated from supragingival dental plaque from adult individuals who had never suffered from dental caries<sup>21</sup>; *S. rubneri* was isolated from throat swab samples of healthy humans<sup>22</sup> and *S. lactarius* from breast milk of healthy women.<sup>23</sup> Members of the *S. mitis* group are known as commensal bacteria of the human oral cavity, gastrointestinal tract and the female genital tract, however, invasive infections might occur when entering the bloodstream.<sup>10</sup> *S. pneumoniae* is associated with bacteremia, meningitis, otitis media and sinusitis; and is the most common cause of community-acquired pneumonia.<sup>10</sup> Commensals such as *S. oralis* and *S. mitis* are major pathogens for infective endocarditis in native or prosthetic heart valves.<sup>10, 24</sup>

Proper identification of species within the *S. mitis* group consisting of phenotypically very closely related species still remains a challenge, in particular for *S. pneumoniae*, *S. pseudopneumoniae*, *S. mitis*, and *S. oralis*. Identification by conventional phenotypic methods including commercial kits as VITEK 2 GP colorimetric card or API rapid 20 Strep strip (bioMérieux, Marcy-l'Étoile, France) are limited<sup>25,26</sup> and newer rapid technologies such as matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) are useful for initial assessment to the *S. mitis* group but frequently do not allow for accurate identification.<sup>27-29</sup> However, there are promising reports elaborating on the differentiation of *S. pneumoniae* from other *S. mitis* group bacteria by MALDI-TOF MS.<sup>30,31</sup> Molecular analysis by the 5'-part of the 16S rRNA gene, which is the gold standard for bacterial identification,<sup>26,32-34</sup> is not sufficiently discriminative to differentiate *S. pneumoniae*, *S. pseudopneumoniae*, *S. mitis* and *S. oralis* due to a >99% sequence homology.<sup>25,35</sup> In the past, several other target genes like *sodA*,<sup>36,37</sup> *rpoB*<sup>38</sup> and *groEL*<sup>39</sup> were proposed for species differentiation. The *recA* gene was recently demonstrated as alternative target to differentiate *S. pneumoniae* from other viridans streptococci.<sup>40</sup> An

interspecies homology of less than 95.8% was shown within a 313-bp part of *recA*, representing a hypervariable region. Additionally, 6 signature nucleotides specific for *S. pneumoniae* were identified within the 313-bp *recA* fragment.

Difficulties in identifying bacteria of the *S. mitis* group to the species level might explain why the novel species *S. tigurinus* was unrecognized and underreported in the past. However, proper identification of those bacteria is important regarding species-specific pathogenicity, efficient

patient management and guidance of appropriate antimicrobial therapy where needed.

#### Microbiological characteristics of

##### *S. tigurinus*.

*S. tigurinus* belongs to Gram-positive cocci arranged in chains and is a non-motile, non-spore-forming and catalase-negative bacterium. Colonies on sheep blood agar are smooth, white to grayish, α-hemolytic and 0.5 to 1 mm in diameter after 24 h incubation at 37°C in aerobic atmosphere. The type strain of the species is AZ\_3a<sup>T</sup> and is deposited in 2 different culture collections: the Culture Collection of Switzerland, CCOS, Wädenswil, Switzerland, under accession number CCOS 600<sup>T</sup>; and the Deutsche Sammlung für Mikroorganismen und Zellkulturen, DSMZ, Braunschweig, Germany, under accession number DSM 24864<sup>T</sup>. The type strain AZ\_3a<sup>T</sup> has a G+C content of the DNA of 40 mol%.<sup>12</sup>

Phylogenetic analysis by the 16S rRNA gene of species of the *S. mitis* group demonstrated that *S. tigurinus* type strain AZ\_3a<sup>T</sup> is clearly distinguished from the other species (Fig. 1). Within the *S. mitis* group, *S. tigurinus* forms a subcluster with *S. pseudopneumoniae*, *S. pneumoniae*, *S. mitis*, *S. oralis* and *S. infantis* (Fig. 1). The species most closely related to the *S. tigurinus* type strain AZ\_3a<sup>T</sup> is *S. mitis* AY485601 with a sequence similarity of 98.6%; the next related species are *S. infantis* AY485603 (98.5%), *S. pseudopneumoniae* AY612844 (98.3%), *S. pneumoniae* AY485600 (98.2%) and *S. oralis* AY485602 (98.1%).<sup>12</sup>

For laboratory diagnostic means, commercial systems such as VITEK 2 colorimetric card (bioMérieux) or MALDI-TOF MS are useful as screening method for assignment of the unknown organism into the *S. mitis* group but do not allow for accurate identification of *S. tigurinus*. Because of the limited database, analysis of the *S. tigurinus* strains by the VITEK 2 colorimetric card (bioMérieux) revealed identification as *S. mitis* / *S. oralis*; whereas MALDI-TOF MS analysis yielded scores of ≥ 2.2 with *S. pneumoniae*, which suggests an identification on species level.<sup>12</sup> Thus, for accurate identification of *S. tigurinus*, sequence analysis

of the partial 16S rRNA gene is mandatory. Recently, a *S. tigurinus* specific real-time TaqMan PCR was developed for highly sensitive and specific detection of *S. tigurinus* directly in clinical samples.<sup>41</sup>

#### Ecological niche of *S. tigurinus* in the human oral microbial flora

The human oral microbiome consists of diverse bacterial phyla, e.g., *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Synergistetes* and *Proteobacteria*.<sup>42,43</sup> Viridans streptococci, e.g., *S. mitis*, are known to be the predominant bacterial species in the human oral cavity and were detected in various dental sites.<sup>42</sup> *S. oralis* is an early colonizing species of the tooth surface and can mediate the first events of biofilm formation. Thus, *S. tigurinus* was also expected to be present in the human oral microbial flora. In a previous report, *S. tigurinus* was not detected in saliva samples, however, the method applied was only based on culturing methods followed by analyses by MALDI-TOF MS.<sup>13</sup> As described before, these methods are limited in accurate identification of *S. tigurinus* thus an underestimation of *S. tigurinus* in the oral cavity seemed likely. By using a more sensitive method, i.e., a *S. tigurinus* specific real-time TaqMan PCR, *S. tigurinus* was detected in half of the individuals investigated.<sup>41</sup> Zbinden et al. analyzed 51 saliva samples and 51 subgingival plaque samples obtained from 51 individuals by the *S. tigurinus* specific real-time TaqMan PCR.<sup>41</sup> *S. tigurinus* was detected both in saliva ( $n = 22$ , 43%) and in subgingival plaque samples ( $n = 18$ , 35%). Overall, in 27 (53%) out of 51 individuals, *S. tigurinus* was detected in the saliva samples and / or in the plaque samples. Saliva consists of bacteria from different oral sites including the subgingival area. Thus it is not surprising that *S. tigurinus* was found in the saliva in a higher frequency than in individually selected subgingival sites. Saliva has been shown to be a suitable biological fluid as alternative to pooled subgingival plaque samples for detection of oral bacteria such as newly identified *Synergistetes*.<sup>44</sup> The presence of *S. tigurinus* in the oral cavity was neither influenced by age nor nicotine consumption.<sup>41</sup>

#### Clinical manifestation of *S. tigurinus*

Members of the microbial flora originating from the oral cavity may be involved in the pathogenesis of systemic infections.<sup>45</sup> Biofilm formation, complex mechanisms with other bacteria or underlying diseases might play a crucial role in the development of invasive infections. To date, all *S. tigurinus* isolates detected from clinical human patient samples were causing severe invasive infections. *S. tigurinus* most frequently caused infective endocarditis ( $n = 7$ ).<sup>13</sup> Over a period of 10 years, 15% of all infective endocarditis cases caused by viridans streptococci were caused by *S. tigurinus*.<sup>13</sup> Other patients developed spondylodiscitis ( $n = 3$ ), bacteremia ( $n = 3$ ), prosthetic joint infection ( $n = 2$ ) and meningitis ( $n = 1$ ) caused by *S. tigurinus*.<sup>12-14</sup> *S. tigurinus* was isolated from normally sterile human body sites, e.g., blood, heart valves, cerebrospinal fluid and periarticular joint biopsy specimens. All patients recovered after appropriate antimicrobial therapy, however, 8 out of 16 patients required surgical interventions. *S. tigurinus* affected not only immunocompromised patients or patients

with underlying conditions such as preexisting cardiac morbidities but also healthy young patients without any comorbidities.<sup>12-14</sup> To date, a specific risk factor profile for the development of invasive infections with *S. tigurinus* could not be established yet due to the limited number of patients.

To note, *S. tigurinus* still might be underreported as causative agent of invasive infections for laboratory diagnostic reasons. Most microbiological diagnostic laboratories rely on identification methods by mass spectrometry, which usually allows rapid identification of bacteria but has a limited discriminative power when analyzing closely related species. Molecular techniques facilitating accurate identification of *S. tigurinus* are not applicable in every routine microbiological laboratory. Yet, the occurrence of *S. tigurinus* in causing invasive infections seems geographically unlimited, since in a recent report of Japan *S. tigurinus* was demonstrated to be the causative agent in 2 cases of infective endocarditis.<sup>46</sup>

*S. tigurinus* is mostly fully susceptible to different antimicrobial classes. Susceptibility to penicillin, gentamicin, vancomycin, levofloxacin, erythromycin and clindamycin was demonstrated.<sup>13</sup> Some *S. tigurinus* strains displayed reduced susceptibility or resistance to tetracycline.<sup>13</sup> Nevertheless, the antibiotic resistance profile should be determined in each invasive isolate as *S. mitis* group species are known for bacterial transformation and can acquire multiple genetic elements containing resistance genes.<sup>47</sup>

#### Association of *S. tigurinus* with oral infections

Although more than 700 species were shown to colonize the oral cavity,<sup>42</sup> evidence suggests that only a few of them, e.g., *Aggregatibacter actinomycetemcomitans* or *Porphyromonas gingivalis*, are associated with the pathogenesis of periodontitis or systemic complications.<sup>48,49</sup> Streptococci were found to be more prevalent in healthy individuals, however, *S. parasanguinis* was proposed to be involved in localized aggressive periodontitis by interaction with the major pathogen *A. actinomycetemcomitans*.<sup>50</sup> Furthermore, *S. mitis* is overrepresented in endodontic infections,<sup>51</sup> the etiology of which likely is polymicrobial.<sup>52</sup>

To date, it is not yet fully understood whether or not *S. tigurinus* is more prevalent in individuals with periodontitis or if it is involved in the development of other oral infections. *S. tigurinus* not only was detected in patients with periodontitis but also in individuals without periodontal diseases.<sup>41,53</sup> Earlier studies have demonstrated that *S. mitis*, which is the closest related species to *S. tigurinus*, is a predominant early colonizing species of dental biofilms.<sup>54</sup> Although *S. mitis* is not a potent inducer of immune responses, it can antagonize the capacity of *A. actinomycetemcomitans* (a key pathogen associated with the localized aggressive form of periodontitis occurring in younger individuals) to stimulate IL-8.<sup>55</sup> Interaction of *S. tigurinus* with *A. actinomycetemcomitans* might be of interest.<sup>56</sup> Since its recent identification, it is not clear whether modifying factors are associated with the presence of *S. tigurinus* in the human oral microbiome and if its detection in the oral cavity has direct clinical implications in systemic diseases.



### Pathogenicity of *S. tigurinus*

Although *S. tigurinus* is a commensal of the human oral cavity as other members of the *S. mitis* group, specific virulence factors which allow for entering the bloodstream and causing severe invasive infections, e.g., infective endocarditis, must be present. In a rat model of experimental endocarditis, *S. tigurinus* was demonstrated to be highly virulent, however, a natural intraspecies variability of different *S. tigurinus* isolates regarding its pathogenicity potential was observed.<sup>57</sup> Most *S. tigurinus* strains had a more than 10-times higher capacity rate to induce aortic infection in rats than *S. gordonii*, a well-known endocarditis pathogen.<sup>58</sup> Moreover, the infectivity rate was similar to the most aggressive infective endocarditis pathogens, i.e., *Staphylococcus aureus* and enterococci.<sup>57</sup> Several virulence determinants were shown to be present in *S. tigurinus*. Phagocytosis of *S. tigurinus* by macrophages significantly was reduced compared to *S. gordonii*. The ability to resist to phagocytosis is a key attribute of invasive streptococci such as *Streptococcus pyogenes*.<sup>59</sup> Adherence and invasion to endothelial cells, a prerequisite for effective host cell internalization, was enhanced in some *S. tigurinus* strains as well as induction of platelet aggregation.<sup>57</sup> Whole-genome analyses of *S. tigurinus* were performed to unravel the genetic background of the pathogenic phenotype; genes of known virulence factors such as exfoliative toxin and fibronectin-binding protein, as well as several phages were identified.<sup>60</sup>

### Small-colony variants of *S. tigurinus*

The occurrence of small-colony variants (SCVs) in oral bacteria, e.g., viridans streptococci has been rather unknown. There are only a few reports describing *S. pneumoniae* mucoid variants and SCVs in biofilms.<sup>61,62</sup> Recently, a prosthetic joint infection caused by *S. tigurinus* SCVs was described.<sup>14</sup> SCVs of bacteria are a pathogenic life form promoting persistent and recurrent infections.<sup>63</sup> SCVs frequently cause infections associated with foreign-body material such as cardiac devices<sup>63,64</sup> or prosthetic joints.<sup>65</sup> Infection by bacterial SCVs may lead to therapy failure and often makes a definite eradication very difficult.<sup>65-67</sup> Therefore, timely diagnosis of these bacterial life variants has a major clinical impact regarding patient management and antibiotic therapy. Morphological and phenotypic characteristics of SCVs are small colony size, atypical colony morphology and reduced growth.<sup>63,68</sup> SCVs of viridans streptococci might have been overlooked in the past as even the wild-type (WT) phenotype of viridans streptococci displays tiny colonies. Moreover, overgrowth by the WT phenotype might complicate the isolation of the SCVs. SCVs often display auxotrophy for hemin, menadione or thymidine due to deficiency in electron transport or thymidine biosynthesis.<sup>63,68</sup> Morphological and biochemical characteristics of SCVs are extensively studied in staphylococci,<sup>63</sup> however, SCVs are found in various genera and species, e.g., enterococci,<sup>69</sup> *Escherichia coli*<sup>70</sup> and *Pseudomonas aeruginosa*.<sup>63</sup> The *S. tigurinus*

SCVs displayed very small, pinpointed colonies, had a considerably reduced exponential growth phase compared to the WT and showed either a very stable or a fluctuating SCV phenotype.<sup>14</sup> The unstable SCV phenotype characterized by a switch from SCVs to revertant normal colonies after a few passages was first recognized in staphylococci.<sup>64</sup> In the *S. tigurinus* SCVs, no auxotrophy for hemin, menadione or thymidine was detected. The ultrastructure of the *S. tigurinus* SCVs were characterized in-depth by transmission electron microscopy analyses showing major alterations in cell separation and morphological abnormalities.<sup>14</sup> Autolysis of *S. tigurinus* SCVs was impaired in such the SCVs displayed an increased spontaneous autolysis compared to the WT but an unexpectedly reduced autolysis under induction with Triton X-100, which is a potent autolysis inducer.<sup>14</sup> Whole-genome sequencing revealed mutations in genes involved in general cell metabolism, cell division, stringent response and virulence, which might partially explain the *S. tigurinus* SCV phenotype.<sup>14</sup>

*S. pneumoniae* SCVs were identified in biofilms,<sup>61</sup> however, whether or not *S. tigurinus* is able to develop a SCV phenotype under such circumstances is not yet known. Biofilm formation of SCV bacteria with specialized functions has been suggested to be a survival strategy, enabling tolerance to a wide variety of environmental conditions.<sup>71</sup> Oral bacteria forming subgingival biofilm communities may lead to development of periodontitis. In the absence of the red complex (*Treponema denticola*, *P. gingivalis* and *Tannerella forsythia*), *S. oralis* was found to dominate the biofilm composition in in-vitro models.<sup>72</sup> Hence in the absence of the red complex, *S. oralis* may display more decisive virulence characteristics within a mature biofilm community. *S. oralis* is a very closely related species of *S. tigurinus*, however, future investigations are warranted to prove if *S. tigurinus* has a similar behavior in subgingival biofilm formation.

## Conclusions

*S. tigurinus* is detected as part of the oral microbiota, including patients with periodontal infection. Still, a cause-effect relationship in oral infections cannot be established by the limited data available. It further needs to be established if *S. tigurinus* can be part of pathogenic oral biofilms, how it associates with other members of the oral microbiota, and whether it is a potent inducer of pathogenic host responses. Whichever the case, however, its presence in the oral cavity and its association with endocarditis warrants special attention for a link between oral and systemic infection.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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