Targeted Killing of *Streptococcus mutans* by a Pheromone-Guided “Smart” Antimicrobial Peptide

Randal Eckert,1 Jain He,2 Daniel K. Yarbrough,2 Fengxia Qi,2 Maxwell H. Anderson,3 and Wenyuan Shi1,2,*

Department of Microbiology, Immunology, and Molecular Genetics1 and School of Dentistry,2 University of California, Los Angeles, California 90095, and C3 Ian, Incorporated, Sequim, Washington 983823

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Within the repertoire of antibiotics available to a prescribing clinician, the majority affect a broad range of microorganisms, including the normal flora. The ecological disruption resulting from antibiotic treatment frequently results in secondary infections or other negative clinical consequences. To address this problem, our laboratory has recently developed a new class of pathogen-selective molecules, called specifically (or selectively) targeted antimicrobial peptides (STAMPs), based on the fusion of a species-specific targeting peptide domain with a wide-spectrum antimicrobial peptide domain. In the current study, we focused on achieving targeted killing of *Streptococcus mutans*, a cavity-causing bacterium that resides in a multispecies microbial community (dental plaque). In particular, we explored the possibility of utilizing a pheromone produced by *S. mutans*, namely, the competence stimulating peptide (CSP), as a STAMP targeting domain to mediate *S. mutans*-specific delivery of an antimicrobial peptide domain. We discovered that STAMPs constructed with peptides derived from CSP were potent against *S. mutans* grown in liquid or biofilm states but did not affect other oral streptococci tested. Further studies showed that an 8-amino-acid region within the CSP sequence is sufficient for targeted delivery of the antimicrobial peptide domain to *S. mutans*. The STAMPs presented here are capable of eliminating *S. mutans* from multispecies biofilms without affecting closely related noncariogenic oral streptococci, indicating the potential of these molecules to be developed into “probiotic” antibiotics which could selectively eliminate pathogens while preserving the protective benefits of a healthy normal flora.

The indigenous microflora found at human mucosal surfaces is critical for acquiring nutrients and providing protective colonization against pathogenic microorganisms (9, 35, 36, 40, 41, 47). When the normal flora is disrupted by any number of factors, the result is often microbial infections at the mucosal surface, many of which affect populations worldwide (32, 44). The lack of a robust immune response at mucosal surfaces has limited the prescribing clinician to conventional antibiotics or antimicrobials for treatment of mucosal infections. Unfortunately for the normal flora, most small-molecule antibiotics have broad spectra of activity, killing benign and pathogenic organisms indiscriminately. This effect often leads to severe antibiotic-associated infections due to the vacated niche available for pathogen colonization. *Clostridium difficile*, *Candida albicans*, and *Staphylococcus aureus* are examples of classical opportunistic pathogens that take advantage of increased niche size after antibiotic treatment (19, 28, 42). The problems resulting from wide-spectrum antibiotic use, combined with the emergence of drug-resistant strains, highlight the fundamental need for new “targeted” antibiotic therapies to combat mucosal pathogens with a minimal impact on normal microflora.

Previous efforts toward achieving target-specific antimicrobial therapy consisted of conjugating antibiotics to monoclonal antibodies (W. Shi, S. L. Morrison, K. Trinh, L. Wims, L. Chen, M. H. Anderson, and F. Qi, U.S. patent application 20030143234) or constructing large fusion proteins with bactericidal and bacterial recognition domains (38, 39). To date, neither method has resulted in functional, effective therapeutics; this is due to the low efficiency of chemical conjugation, instability of large proteins, or high cost of production.

Recently, we developed a new class of targeted antimicrobials, called specifically (or selectively) targeted antimicrobial peptides (STAMPs). Constructed from short peptides that can be chemically synthesized with high yields in vitro, STAMPs contain a targeting peptide domain fused to an antimicrobial peptide domain; despite being conjoined, these domains remain functionally independent. As a result, STAMPs have increased killing potency, selectivity, and kinetics against targeted bacteria (15). In our previous report, we used a de novo rationally designed targeting peptide for STAMP construction. In this study, we explored the possibility of co-opting a natural bacterial pheromone (competence stimulating peptide [CSP]) to serve as the targeting peptide domain in a STAMP designed for an oral bacterial pathogen, *Streptococcus mutans* (22, 23, 30).

*S. mutans* has been implicated as a primary pathogen involved in the formation of dental caries (7, 12, 24–26), one of the most prevalent and costly diseases associated with oral mucosal surface infections (4, 5). Despite the presence of over 500 species of indigenous oral flora (1), dental caries result from the overgrowth of a handful of cariogenic pathogens, including *S. mutans*. In order to eliminate or reduce dental caries, numerous studies have focused, through various means, on the prevention of *S. mutans* colonization. However, efforts such as vaccination have yet to yield effective results, and not...
only is the outright sterilization of the oral cavity impossible but efforts to achieve such sterilization have also been associated with secondary infections and resistance evolution (19, 28, 45). We reasoned that the STAMP technology described above could selectively eliminate *S. mutans* without compromising the protective colonization provided by the indigenous flora, thereby preventing *S. mutans* re-colonization and carries progression. Here we report the successful construction and characterization of a set of CSP-derived STAMPs against *S. mutans*.

**MATERIALS AND METHODS**

**Strains and growth conditions.** All *S. mutans*, *S. gordonii* Challis (DL1), and *S. sanguinis* NY101 strains were grown in Todd-Hewitt (TH) broth medium (Fisher) at 37°C under anaerobic conditions (80% N₂, 10% CO₂, 10% H₂). *S. mutans* strains UA159 (2), ATCC 25175, and T8 (43) are wild-type clinical isolates, while *comD* is a knockout mutant that was constructed previously from the wild-type UA140 background (37). Luciferase-expressing *S. mutans* strain JM11 was constructed from strain UA140 as described previously (29).

**Synthesis and purification of peptides.** All peptides listed in Table 1 (also see Table 3) were synthesized using double-coupling cycles by standard 9-fluorenylmethoxycarbonyl (Fmoc) solid-phase synthesis methods (431A peptide synthesizer [Applied Biosciences] or Apex96 [Advanced Chemtech]) as described previously (15). Completed peptides were cleaved from the resin with 95% trifluoroacetic acid with appropriate scavengers and purified by reverse-phase high-performance liquid chromatography (ACTA purifier; Amersham) to 90% to 95% purity. Peptide molecular mass was determined by matrix-assisted laser desorption ionization mass spectrometry. Peptides C16G2, G2, and M8G2 were synthesized using amidated C termini and Fmoc-OtBu-Rink Amide MBHA resin (Anaspec). All other peptides were synthesized with the appropriately substituted Wang resins.

**Fluorescent labeling of peptides and fluorescence microscopy.** Aliquots of CSP, CSP-fragment peptides (see Table 3), and C16G2 were labeled with carboxyfluorescein (Sigma) as described previously (15, 16). After peptide cleavage but prior to the bacterial labeling assay, fluorescence intensity values per micromole peptide were checked fluorimetrically (VersaFluor, Fluor-BioRad) and standard curves for each fluorophore were generated. Luciferase-expressing *S. mutans* UA159 biofilms were grown with 0.5% (wt/vol) sucrose. Plates were then centrifuged briefly to pellet the cells, and bacteria were incubated for 3 to 4 h at 37°C for biofilm formation. After incubation, the supernatants were then removed, and biofilms were treated with 25 μM peptide in 1× PBS or 1× PBS alone for 1 min. The peptide solution was then removed, and 100 μl TH medium was added to further dilute any remaining peptide. To minimize biofilm loss, cells were briefly centrifuged after TH medium addition, after which the supernatants were removed and fresh medium plus appropriate sugars were added. Cells were then incubated anaerobically at 37°C, and biofilm growth was monitored over time by measuring absorbance at OD₅₅₀ with a microplate spectrophotometer (Benchmark Plus; Bio-Rad).

**Evaluation of antimicrobial activity against bacterial biofilm in saliva.** For these experiments, we employed methods similar to those previously described (4, 8). A day prior to the assay, saliva was collected and pooled from five adult volunteers in the laboratory, diluted 1:4 in TH broth, and centrifuged at 2,000 × g for 10 min. The supernatants were then filter sterilized (Nunc) (0.2 μm filter) and stored at 4°C. A portion of pooled saliva was also diluted 1:2 in 1× PBS and processed as before. On the day of the assay, overnight cultures of JM11 and other oral streptococci were normalized to an OD₅₅₀ of 1.0 and −3 × 10⁶ CFU/ml of each species were added to 10 ml of the TH medium-diluted saliva. Sucrose, mannose, and glucose (*S. mutans* UA159 biofilms were grown with 0.5% wt/vol sucrose. Plates were then centrifuged briefly to pellet the cells, and bacteria were incubated for 3 to 4 h at 37°C for biofilm formation. After incubation, the supernatant was carefully removed and biofilms were treated with 25 μM peptide in 1× PBS or 1× PBS alone for 1 min. The peptide solution was then removed, and 100 μl TH medium was added to further dilute any remaining peptide. To minimize biofilm loss, cells were briefly centrifuged after TH medium addition, after which the supernatants were removed and fresh medium plus appropriate sugars were added. Cells were then incubated anaerobically at 37°C, and biofilm growth was monitored over time by measuring absorbance at OD₅₅₀ with a microplate spectrophotometer (Benchmark Plus; Bio-Rad).

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**RESULTS**

**Design and construction of C16G2, a CSP-derived STAMP against *S. mutans*.** The initial CSP-derived STAMP was constructed by synthesizing full-length *S. mutans*-specific CSP (21 amino acids) with the antimicrobial peptide G2 (16 amino acids, derived from the wide-spectrum antimicrobial peptide novospirin G10) (15) at either the C terminus or the N terminus. Biological testing of these STAMPs did not reveal any
antimicrobial activity (data not shown). We reasoned that steric hindrance caused by the full-length CSP might be inhibiting G2 antimicrobial function. Therefore, since a shorter targeting domain could be advantageous, we theorized that the C-terminal 16 amino acids of CSP, called CSP\(_{C16}\) (which in previous studies was shown to still have pheromone activity) (37), could be used as a substitute for CSP. Peptides containing CSP\(_{C16}\) at either the N or C terminus of G2, with different linker regions of flexible amino acids in between, were then synthesized and screened for their antimicrobial activities (data not shown). From among the potential STAMPs, C16G2, which consisted of (from the N to C terminus) CSPC16, a short peptide linker (GGG), and G2 (Table 1), was selected for further study due to its improved MIC, greatly enhanced killing kinetics, and selectivity against \(S.\) \textit{mutans} (compared to that of G2 alone), as described below.

**C16G2 has enhanced antimicrobial activity and specificity against planktonic \(S.\) \textit{mutans} cells.** To evaluate the antimicrobial activity and general specificity of C16G2, MIC tests were performed against a panel of bacterial species, including various strains of \(S.\) \textit{mutans} and closely related oral streptococci (18). As shown in Table 2, the MICs for C16G2 ranged from 3 to 5 \(\mu\)M for all \(S.\) \textit{mutans} strains tested, a four- to fivefold increase in antimicrobial activity over that seen with the parental antimicrobial peptide G2 (12 to 20 \(\mu\)M). In comparison, we observed little (twofold or less) difference in susceptibility between G2 and C16G2 against \(S.\) \textit{gordonii} and \(S.\) \textit{sanguinis}.

Previously, we showed that an anti-\textit{Pseudomonas} STAMP did not show much improvement in MIC after 24 h incubation but displayed greatly enhanced killing kinetics and specificity (compared to the untargeted parental antimicrobial peptide) against the targeted bacteria during a short time exposure (15). Therefore, we performed comparative experiments to examine the killing ability of C16G2 and G2 for its targeted and untargeted bacteria after a short time exposure. As shown in Fig. 1, with 1 min of exposure, C16G2 was over 20-fold more active against its targeted bacterium, \(S.\) \textit{mutans}, in comparison to G2, whereas it exhibited a level of activity similar to that seen with G2 against other oral streptococci tested. These findings provided the first indications that the addition of the CSP\(_{C16}\) targeting domain to G2 had resulted in an antimicrobial with selective activity against \(S.\) \textit{mutans} but against not other closely related oral streptococci.

**C16G2 is also active against biofilm cells.** \(S.\) \textit{mutans} bacteria predominantly exist in a biofilm growth state in vivo. As it is well known that biofilm-associated cells are 100- to 1,000-fold more resistant to antibiotics (14, 17, 46), we tested whether C16G2 still has activity against \(S.\) \textit{mutans} biofilms in vitro. For these experiments, biofilm-associated \(S.\) \textit{mutans}, \(S.\) \textit{gordonii}, or \(S.\) \textit{sanguinis} bacteria were treated with 25 \(\mu\)M C16G2, G2, CSP, CSP\(_{C16}\), or 1\(\times\) PBS for 1 min and washed, and their regrowth was monitored over time. As shown in Fig. 2, \(S.\) \textit{gordonii} or \(S.\) \textit{sanguinis} biofilms exposed to any of the peptides tested grew similarly to untreated biofilms after peptide addition and removal (Fig. 2A and B). In contrast, \(S.\) \textit{mutans} strain UA159 (Fig. 2C), as well as strains T8 and 25175 (data not shown), was severely inhibited by treatment with C16G2 but was unaffected by treatment with the other peptides. These results indicate that C16G2 can function as an anti-\textit{S. mutans} STAMP in a biofilm environment with only a short period of exposure (1 min), a time period which is relevant for clinical treatments of the oral cavity (6, 27, 48).

**C16G2 can selectively eliminate \(S.\) \textit{mutans} from a mixed species biofilm.** In addition to growing as biofilm in vivo, \(S.\) \textit{mutans} bacteria are also constantly bathed in saliva as they adhere to the tooth surface. Therefore, we examined whether C16G2 could selectively kill \(S.\) \textit{mutans} under these conditions.


\begin{table}
\centering
\begin{tabular}{l|c|c|c|c|c|c}
\hline
\textbf{Strain} & \textbf{MIC (\(\mu\)M)} & \\
 & CSP & CSP\(_{C16}\) & G2 & C16G2 & CSP\(_M8\) & M8G2 \\
\hline
\textit{S. mutans} & & & & & & \\
UA159 & 50.8 \(\pm\) 9.3 & >60 & 12.1 \(\pm\) 4.5 & 3.0 \(\pm\) 1.6 & >60 & 3.25 \(\pm\) 1.9 \\
25175 & >60 & >60 & 14.8 \(\pm\) 2.0 & 3.8 \(\pm\) 0.3 & >60 & 3.5 \(\pm\) 0.5 \\
T8 & >60 & >60 & 14.2 \(\pm\) 1.5 & 3.7 \(\pm\) 0.2 & >60 & NT* \\
comD & >60 & >60 & 15.3 \(\pm\) 4.2 & 5.1 \(\pm\) 2.4 & >60 & 4.0 \(\pm\) 2.0 \\
\hline
\textit{Non-\textit{S. mutans}} & & & & & & \\
\textit{S. gordonii} & >60 & >60 & 41.3 \(\pm\) 14.0 & 23.5 \(\pm\) 7.8 & >60 & 20 \(\pm\) 5.0 \\
\textit{S. sanguinis} & >60 & >60 & 33.6 \(\pm\) 7.5 & 19.1 \(\pm\) 4.0 & >60 & 15 \(\pm\) 2.5 \\
\hline
\textit{a} MICs represent averages of at least 3 independent experiments with standard deviations. \\
\textit{b} NT, not tested.
\end{tabular}
\caption{MICs of G2-containing STAMPs and STAMP components against bacteria}
\end{table}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{Selective killing activity of C16G2 against \textit{S. mutans}, \textit{S. mutans}, \textit{S. gordonii}, and \textit{S. gordonii} planktonic cells were exposed to 25 \(\mu\)M of the STAMP C16G2, or its untargeted parent antimicrobial peptide G2, for 1 min. Surviving CFU per milliliter were detected and compared. Data represent averages of the results of at least three independent experiments.}
\end{figure}
In these experiments, two species of noncariogenic oral streptococci (\textit{S. gordonii} and \textit{S. sanguinis}) were mixed with \textit{S. mutans} JM11, a strain harboring a transcriptional fusion between luciferase (\textit{luc}) and the promoter for the constitutively active gene lactate dehydrogenase (\textit{ldh}), which has the same susceptibility to C16G2 as the wild-type UA159 (data not shown). JM11 has been previously utilized to measure the fitness of \textit{S. mutans} populations, and decreasing RLU production was shown to strongly correlate with reduced cell viability (29). The mixed-species biofilms were formed with saliva, and then peptides (25 μM) suspended in saliva were added for 5 min and removed, and the posttreatment growth of the biofilm was further monitored. The number of viable \textit{S. mutans} cells within the biofilm was quantified in parallel by luciferase expression. We found that C16G2 was able to dramatically reduce the \textit{S. mutans} population within the mixture (reflected in the low level of luciferase activity) after 5 min of exposure compared to the results seen with CSP\textsubscript{C16} and G2 (Fig. 3). Interestingly, even after 120 min posttreatment, the total number of \textit{S. mutans} within the mixture remained low (Fig. 3). Taken together, these results indicate that a short exposure of C16G2 is capable of selectively inhibiting the growth of \textit{S. mutans} within a multispecies biofilm and in the presence of saliva for a minimum of 2 h without harming bystander bacteria or affecting the overall health of the biofilm.

Enhanced antimicrobial activity of C16G2 is related to targeted ComD-independent binding of CSP\textsubscript{C16} to \textit{S. mutans}. To further explore the mechanism of C16G2-enhanced activity against \textit{S. mutans}, we fluorescently labeled CSP\textsubscript{C16} and C16G2 and tested their ability to bind to \textit{S. mutans} and other streptococci. Consistent with observed killing activity, we found that CSP\textsubscript{C16} and C16G2 could specifically bind to \textit{S. mutans} with a very short time exposure (1 to 2 min) but not to other oral streptococci (data not shown).

We also tested the effect of C16G2 on the \textit{comD} mutant, as previous genetic studies suggested that CSP may interact with ComD to activate DNA competence in \textit{S. mutans} (23). To our surprise, similar MICs were observed for UA159 and the \textit{comD} strain (Table 2). Consistent with this observation, we also found that fluorescently labeled CSP\textsubscript{C16} and C16G2 (data not shown) bound to UA159 and the \textit{comD} mutant in a similar manner, indicating that the specific binding ability of CSP to \textit{S. mutans} is independent of the presence of ComD.

An 8-amino-acid sequence within C16 is required for species-specific recognition. To determine whether there was a region within the CSP\textsubscript{C16} sequence that was responsible for \textit{S. mutans}-specific binding, we synthesized a series of fluorescently labeled CSP\textsubscript{C16} fragments and analyzed their ability to bind to \textit{S. mutans}. The following strategies were utilized in dissecting the CSP\textsubscript{C16} sequence (Table 3). First, a series of fragments were constructed by generating deletions of three or four amino acids, from the N to C terminus, across the CSP\textsubscript{C16} sequence (C16-1 to C16-5). Peptides lacking larger portions of the C or N terminus of CSP\textsubscript{C16} (C16-6 to C16-12) were also synthesized. Additionally, peptides with Arg to Asn substitu-
Terminal deletions
C16-6 TFFRLFNR----- + ++
C16-7 ------RNFRT----- +
C16-8 -------RSFTQALGK --
C16-9 TFFR------- +
C16-10 TFFRFR----- +
C16-11 TFFRFRFR----- +
C16-12 (CSPM8) TFFRFN----------- + ++
Substitutions
C16-13 TGGGRGNSGTFQLGK --
C16-14 TGFNLFNSFPTQLGK --
Alanine scanning
C16-15 AAAALFNRSTFTQALGK --
C16-16 TFFRAAAAFSTQALGK +
C16-17 TFFRFFAAAALGK +
C16-18 TFFRFNFRTFGAAAA + ++

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**FIG. 4.** Activity of M8G2 against oral bacteria in biofilms. *S. mutans* (A) or *S. sanguinis* (B) single-species biofilms were mock treated or exposed to 25 μM M8G2 (as specified in the figure). After removal of the STAMP and the addition of fresh medium, biofilm recovery was monitored over time by monitoring absorbance at OD600. The data represent the averages of the results of three independent experiments.
the STAMPs was readily apparent: both C16-33 and M8-33 were capable of retarding \textit{S. mutans} biofilm growth after a short exposure (Fig. 5A), while cultures of \textit{S. sanguinis} were not affected by STAMP administration (Fig. 5B). These results indicate a clear enhancement of STAMP activity selective for \textit{S. mutans} biofilms.

**DISCUSSION**

In this study we successfully synthesized and evaluated a series of STAMPs which exhibited specificity for \textit{S. mutans} and not other oral streptococci. The STAMPs were designed for \textit{S. mutans}-selective activity by incorporating portions of a natural pheromone produced by these cariogenic bacteria (CSP) as the targeting domain within the linear STAMP peptide. By exclusively utilizing short (<3 kDa) linear peptides for the targeting and antimicrobial regions, we were able to rapidly synthesize and isolate the complete STAMP molecule in one piece via solid-phase chemical methods, a distinct advantage over the recombinant expression and difficult purification routes necessary to construct the large (>70 kDa) protein-based targeted antimicrobials that have been described previously (38, 39). Additionally, the flexibility provided by synthetic routes enabled us to easily increase STAMP diversity by switching between different combinations of targeting domains (CSP\textsubscript{M8} and CSP\textsubscript{C16}) and killing domains (G2 and S6L3-33) when constructing STAMPs for use against \textit{S. mutans}, a task that would otherwise require tedious cloning procedures.

Our data suggest that CSP\textsubscript{C16}-\textit{S. mutans} binding is species specific but is not dependent on the ComD surface receptor. Furthermore, the CSP\textsubscript{M8} sequence within CSP appears to be sufficient for selective \textit{S. mutans} binding. These results suggest that a natural \textit{S. mutans}-specific targeting sequence is present within this pheromone that may bind to an alternative receptor (lipid, carbohydrate, or protein) on the bacterial surface prior to interaction with ComD. Biologically, the CSP\textsubscript{M8} sequence may function to “sequester” CSP molecules on the \textit{S. mutans} surface, thereby aiding in acquisition of a communication signal for recognition by sensor kinases. Further studies are under way to determine whether minimal binding sequences exist in other species-specific pheromones and whether these sequences are themselves capable of stimulating sensor kinases. Interestingly, CSP\textsubscript{M8} alone appears unable to regulate competence (R. Eckert, F. Qi, and W. Shi, unpublished data).

As shown in Fig. 5, we were able to synthesize CSP\textsubscript{C16} and CSP\textsubscript{M8} in combination with an alternative antimicrobial peptide (S6L3-33) without the loss of \textit{S. mutans}-selective killing ability. This finding further validates the notion that the STAMP targeting and antimicrobial domains function independently and are capable of being linked in different combinations without the loss of activity. This suggests that future STAMP construction will be an unlimited “tunable” process whereby a myriad of combinations of antimicrobial, linker, and targeting domains can be synthesized in order to select a STAMP with the best specific activity. Furthermore, bacterial STAMP resistance (should it evolve) (34) could be easily overcome by switching to alternative, functionally analogous STAMP components, as was done with G2 and S6L3-33 in this study. Additionally, peptide pheromones are widely utilized by pathogenic bacteria (13, 21), especially gram-positive organisms (11, 33), and therefore represent a large and growing pool from which future targeting peptides could be selected for STAMP construction.

C16G2, M8G2, C16-33, and M8-33 displayed robust specific activity against targeted \textit{S. mutans} bacteria in planktonic cultures and in biofilms with both single and multispecies, suggesting that we were able to construct a set of functional STAMPs that can discriminate between \textit{S. mutans} and other noncariogenic oral streptococci. This selective activity, combined with the low cytotoxicity of these peptides (R. Eckert, I. McHardy, J. He, and W. Shi, unpublished data), indicates that they may be useful for anticaries therapeutic development. Currently, treatments for \textit{S. mutans} infection include abstinence from dietary sugars, mechanical removal of the dental plaque, and general biocide mouthwashes. While all are tem-

**TABLE 4. MICs of STAMPs constructed with the S6L3-33 antimicrobial region**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>MIC ± SD (μM) for indicated strain*</th>
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<tbody>
<tr>
<td></td>
<td>UA159</td>
</tr>
<tr>
<td>S6L3-33</td>
<td>7.0 ± 3.0</td>
</tr>
<tr>
<td>C16-33</td>
<td>2.5 ± 2.1</td>
</tr>
<tr>
<td>M8-33</td>
<td>2.5 ± 2.0</td>
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*MICs represent averages ± standard deviations of the results of at least three independent experiments.

**FIG. 5.** Biofilm-inhibitory activity of S6L3-33 and S6L3-33-containing STAMPs. Single-species biofilms of \textit{S. mutans} (A) or \textit{S. sanguinis} (B) were treated with M8-33, C16-33, or S6L3-33 alone (as specified in the figure) for 1 min. After agent removal and stringent washing, the regrowth of the biofilms was tracked over 4 h by measuring absorbance at OD\textsubscript{600} after the addition of fresh medium. The data represent average values obtained from the results of at least three independent assays.
porarily effective to various degrees, the unavoidable loss of normal flora that occurs with mechanical removal or general antibiotic treatment allows S. mutans to reestablish a niche in the oral cavity without difficulty (10, 20, 31). Therefore, a STAMP with S. mutans-selective killing ability would be an ideal solution and could allow the normal flora to outgrow affected S. mutans populations. Such an “antibiotic-probiotic” therapeutic could help prevent dental caries progression and the high health care costs associated with this disease (3).

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