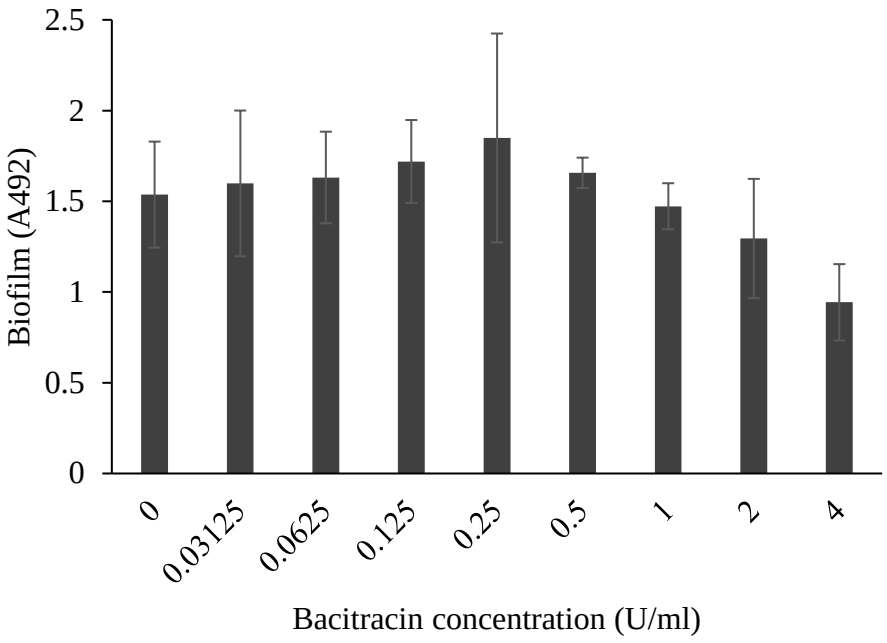


Fig. S1 Biofilm formation assay in TSBs with various concentrations of bacitracin. The effect of bacitracin at sub-MIC on polysaccharide-dependent biofilm by the WT strain. To evaluate the effect of bacitracin, we performed biofilm formation assay using TSBs medium which was known as a biofilm-inducible medium. At concentrations below MIC (4 U/ml), there was no effect on biofilm formation. The data indicate mean ± standard deviation (SD) of three independent experiments. The data were analyzed with one-way ANOVA and a Bonferroni's posttest.

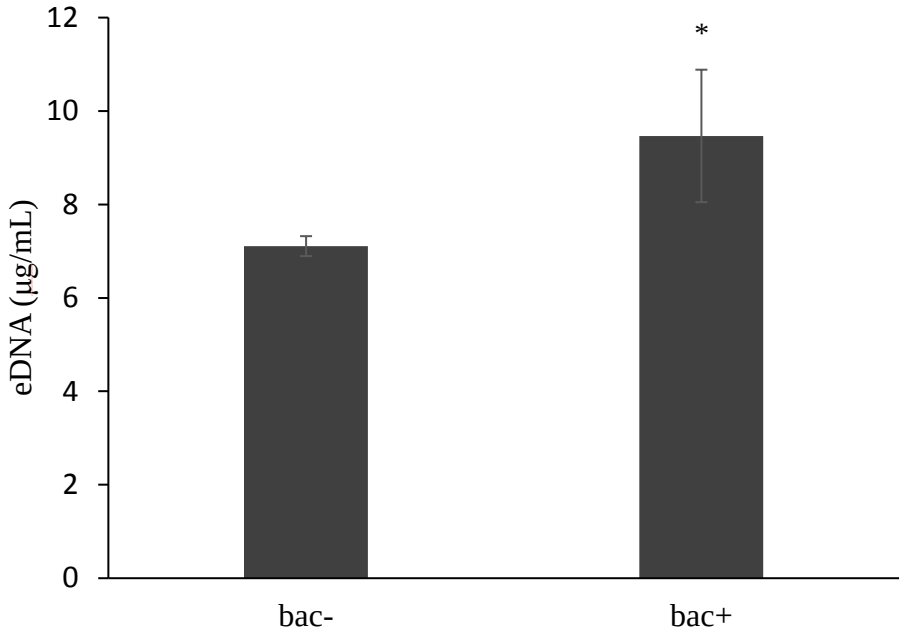


Fig. S2 Comparison of eDNA production of the WT strain in the presence and absence of bacitracin. We determined the amount of eDNA produced by 5 h-cultivation in TSBg without bacitracin (bac-) and with 0.5 U/ml bacitracin (bac+). eDNA production was increase in the presence of bacitracin compared with in the absence of bacitracin. The data indicate mean ± SD of three independent experiments. The asterisks indicate a significant difference between two groups (Student's t-test; *: $p < 0.05$).

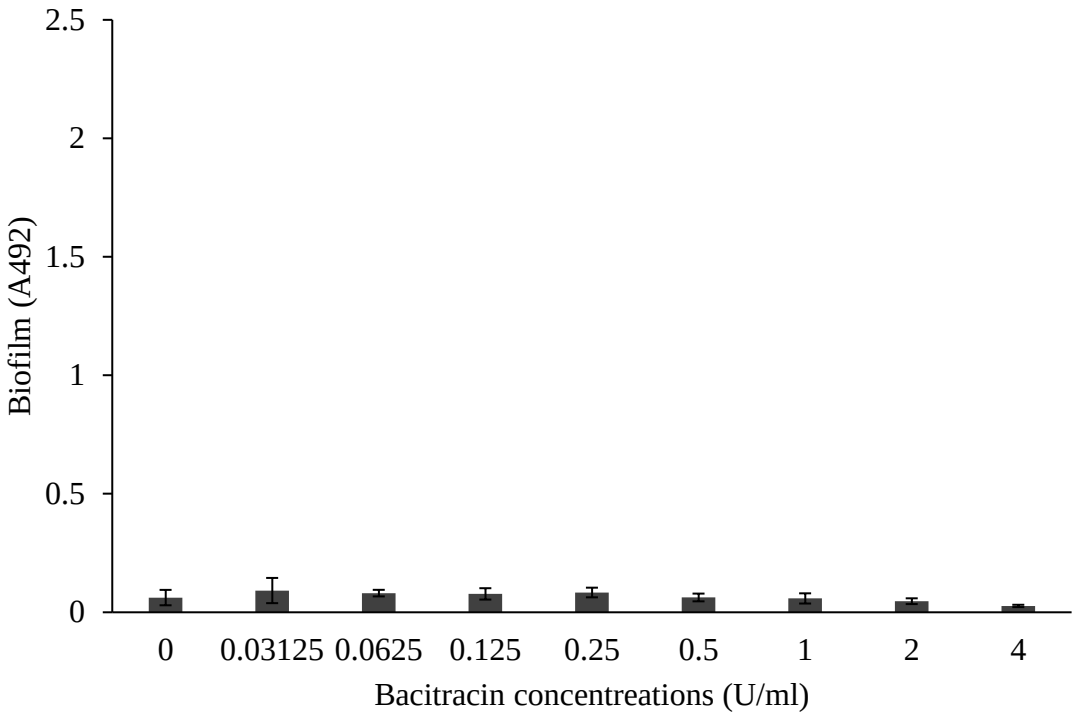


Fig. S3 Biofilm formation assay in SDMg with various concentrations of bacitracin. Considering that sucrose contained in TSB was involved in the biofilm formation, biofilm formation assay was performed in SDMg medium which was a sucrose-free medium. At all bacitracin concentrations, biofilm formation was not induced. Therefore, it was indicated that the sucrose contained in TSB was essential for biofilm induction in the presence of bacitracin at sub-MIC. The data indicate mean ± SD of three independent experiments. The data were analyzed with one-way ANOVA and a Bonferroni's posttest.

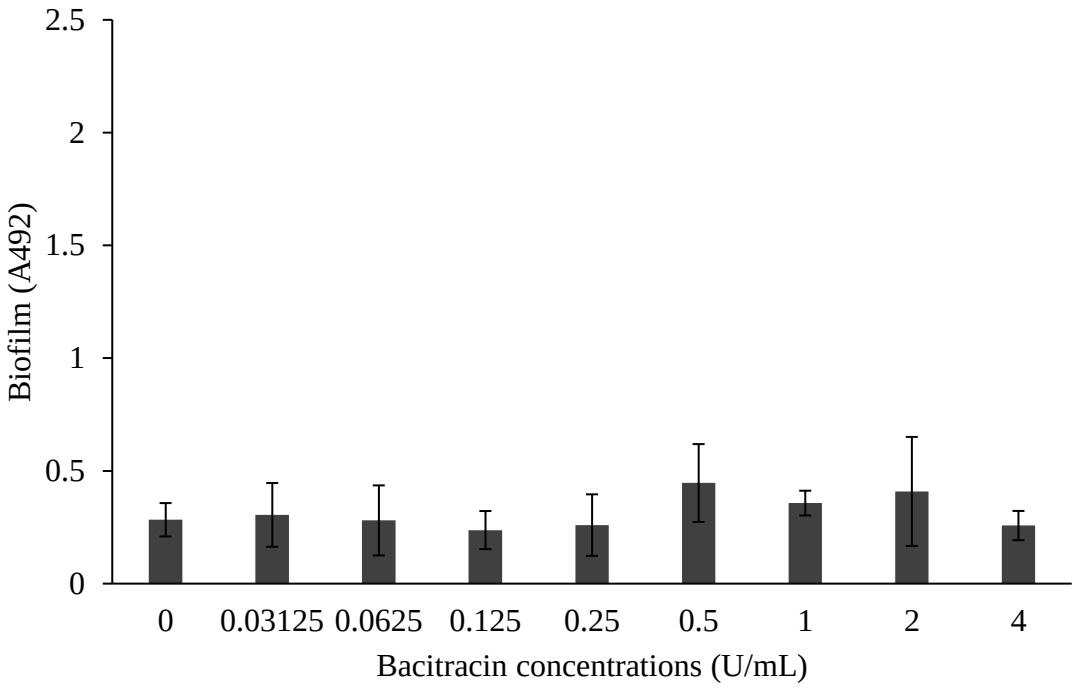


Fig. S4 Biofilm formation assay using the Δ*brpA* mutant strains in TSBg with various concentrations of bacitracin. We investigated whether the biofilm formation was affected when *brpA*, associated with regulation of cell envelop stress, was deleted. The data indicate mean ± SD of three independent experiments. The data were analyzed with one-way ANOVA and a Bonferroni's posttest.

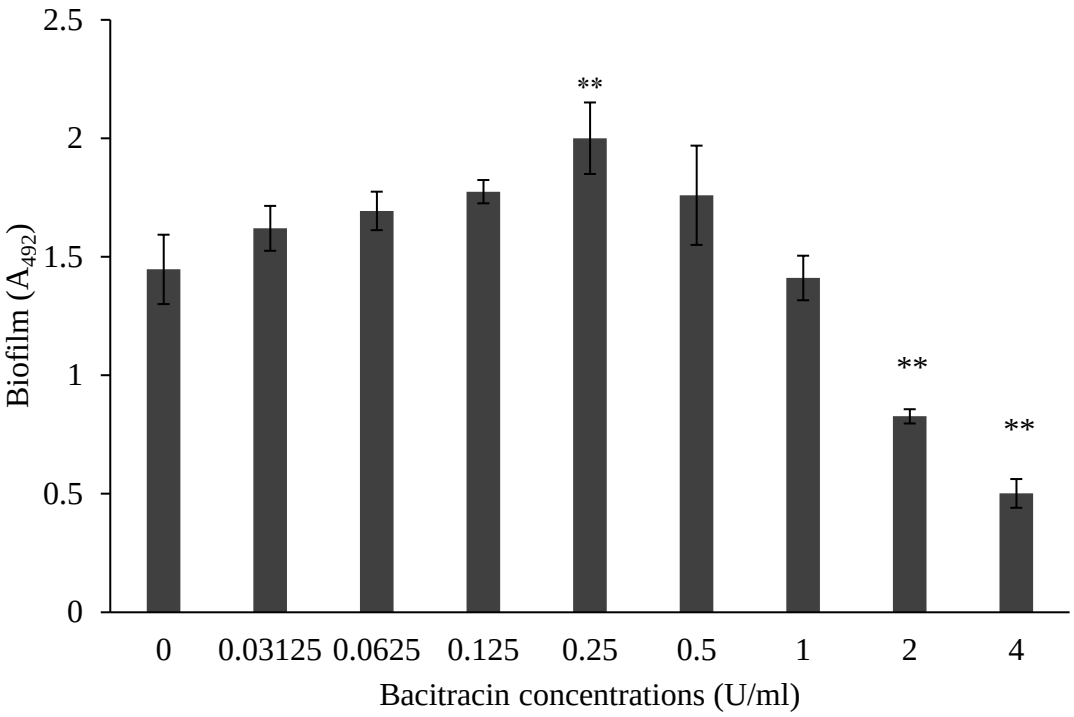
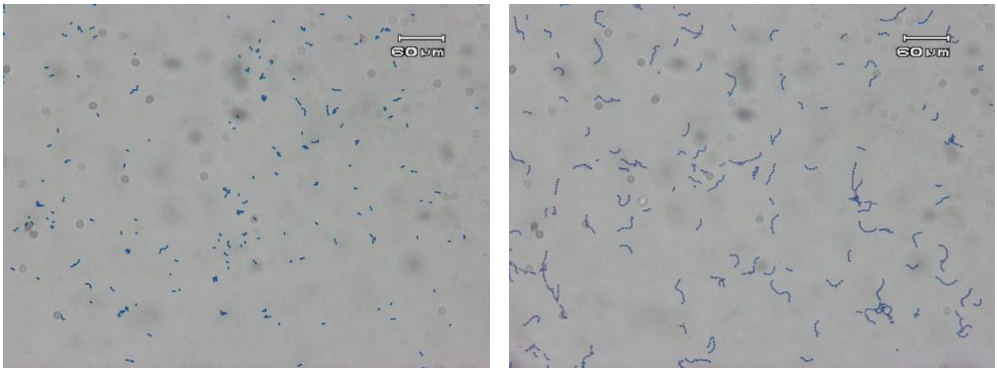


Fig. S5 Biofilm formation assay of the Δ*brpA* mutant strain in TSBs with various concentrations of bacitracin. We investigated whether deletion of *brpA* affect polysaccharide-dependent biofilm formation. The data indicate mean ± SD of three independent experiments. The data were analyzed with one-way ANOVA and a Bonferroni's posttest. The asterisks indicate a significant difference (**: $p < 0.01$, *: $p < 0.05$).



WT Δ*brpA*

Fig. S6 Comparison of cell morphology of the WT strain and the Δ*brpA* mutant strain. The cells were cultivated in TSBg with 0.5 U/ml bacitracin for 5 h and performed microscopic analysis by Gram-staining. In the Δ*brpA* mutant. The Δ*brpA* mutant strain displayed long-chain phenotype compared with the WT strain.

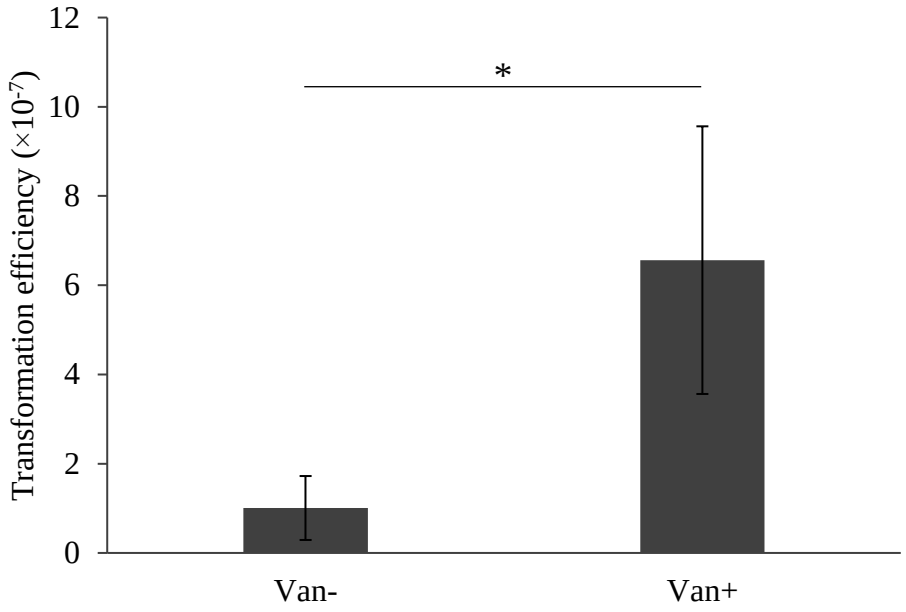


Fig. S7 Transformation assay in the absence and presence of vancomycin. *S. mutans* was cultured in TSBg containing 2.5 μg/ml of pDL278 plasmid carrying a spectinomycin resistance gene. Vancomycin at a concentration of 0.5 μg/ml (1/2 x MIC), which most strongly induced biofilm formation, was added to the medium (Van+). After culturing for 5 h, the cells cultured on Mitis Salivarius agar plate with or without spectinomycin for 36 h. We measured the number of CFUs and determined the transformation efficiency. The data are the mean ± SD of three independent experiments. The asterisks indicate a significant difference between two groups (Student's t-test; *: $p < 0.05$, vs Van-).