Establishing a genetic system for ecological studies of
\textit{Streptococcus oligofermentans}

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\textbf{Abstract}

\textit{Streptococcus oligofermentans} is a newly characterized species belonging to the mitis group of oral streptococci. So far no correlation has been demonstrated between \textit{S. oligofermentans} and dental caries. Furthermore, a reverse correlation has been observed between the number of \textit{S. oligofermentans} and the number of \textit{Streptococcus mutans}, a major cariogenic pathogen, in the oral cavity. These properties suggest that \textit{S. oligofermentans} may have a potential to be used as a ‘probiotics’ for caries prevention. In this study, we aim to establish a genetic system in \textit{S. oligofermentans} to further study the biology of this new species. Using homologous regions of the comCDE locus in other streptococci, the comC gene was isolated and sequenced. A synthetic competence-stimulating peptide (CSP) was synthesized and shown to be able to effectively induce competence in \textit{S. oligofermentans}. This CSP-induced transformation system in \textit{S. oligofermentans} was used to construct green fluorescent protein (gfp) and luciferase (luc) reporter systems, both of which are driven by the lactate dehydrogenase (ldh) promoter. These reporter systems were further shown to be highly expressed in planktonic and biofilm cells, suggesting that these reporter systems can be used in future ecological studies of \textit{S. oligofermentans}.

\textbf{Introduction}

\textit{Streptococcus oligofermentans} is a newly characterized species belonging to the mitis group of oral streptococci (Tong \textit{et al.}, 2003). It was initially isolated from the dental plaques of carious-free humans in China. Epidemiological studies have not found any correlation between \textit{S. oligofermentans} and dental caries, suggesting that this oral streptococcus may be an oral commensal bacterium. Furthermore, a reverse correlation has been observed between the number of \textit{S. oligofermentans} and dental caries (Loesche, 1986). Recent studies in our laboratories demonstrated that this reverse correlation might be attributable to the production of large amount of H\textsubscript{2}O\textsubscript{2} by \textit{S. oligofermentans} that could suppress the growth of \textit{S. mutans} (data will be published elsewhere). Moreover, \textit{S. oligofermentans} produces less acid while growing under laboratory conditions and only weakly demineralizes hydroxyapatite in \textit{in vitro} experiments. These properties suggest that \textit{S. oligofermentans} may have a potential to be used as a ‘probiotics’ for caries prevention. However, currently nothing is known about the genetics and biology of this bacterium, despite limited physiological studies (Tong \textit{et al.}, 2003). To better understand the biology of this new bacterial species, in this study, we aim to establish a genetic system in \textit{S. oligofermentans} to study further its ecological role.

Genetic systems have been well established in other members of the mitis group streptococci. In general, most (if not all) members in this group possess a natural transformation system (competence) (Tomasz \& Mosser, 1966; Morrison \& Baker, 1979; Havarstein \textit{et al.}, 1997). Competence is a transient physiological state at which the bacteria develop a capacity to take up exogenous DNA. The DNA can be integrated into the host genome by homologous recombination or stay as an autonomous entity if an origin of replication is included (Lorenz \& Wackernagel, 1994). In the mitis group streptococci, natural competence is controlled by a quorum-sensing system called comCDE (Lunsford \& London, 1996; Havarstein \textit{et al.}, 1997; Kleerebezem \textit{et al.}, 1997). comC encodes the signaling peptide competence stimulating peptide (CSP) and comDE encode the two-component signal transduction system responsible for transducing signals mediated by CSP binding (Cheng \textit{et al.}, 1997; Lee \& Morrison, 1999). Previous studies have shown
that the organization of comCDE locus is highly conserved among members of the mitis group streptococci (Havarstein et al., 1997). Therefore, in this study, we used degenerate primers designed based on the sequences of comD and arginine tRNA gene of Streptococcus pneumoniae, Streptococcus gordonii and Streptococcus mitis to amplify the corresponding region in S. oligofermentans. Through sequencing, the CSP peptide sequence was identified and a functional CSP was chemically synthesized. Using the synthetic CSP, we established a genetic transformation system in S. oligofermentans, through which, two reporter systems that could be used in ecological studies of S. oligofermentans were constructed.

### Materials and methods

#### Bacterial strains and culture conditions

Bacterial strains and plasmids used in this study are listed in Table 1. All Streptococcus strains were routinely grown in brain heart infusion (BHI) medium (Difco, BD) or on BHI agar plates at 37 °C in a candle jar. For biofilm assay, bacteria were grown in Todd Hewitt Broth (TH; Difco, BD) plus 1% glucose and 0.5% sucrose. BHI medium supplemented with 100 ng DNA, 0.4 µM each of primer, 0.1 mM dNTP, 10 × PCR buffer and 0.1 U Taq polymerase (Takara Company, Dalian, China). PCR was performed at 95 °C/5 min, five cycles at 94 °C/30 s, 45 °C/1 min, and 72 °C/1 min, 30 cycles at 94 /30 s, 50 °C/1 min, and 72 °C/1 min, then one cycle at 72 °C/10 min. The comC gene fragment produced was cloned into pUCm-T and sequenced from both ends with the original DNA as PCR template with a thermo-cycling machine (Thermolyhaid). The 25 µL PCR reaction mixture contained 100 ng DNA, 0.4 µM each pair of primer, 0.1 mM dNTP, 10 × PCR buffer and 0.1 U Taq polymerase (Takara Company, Shanghai, China). PCR was performed at 95 °C/5 min, five cycles at 94 °C/30 s, 45 °C/1 min, and 72 °C/1 min, 30 cycles at 94 °C/30 s, 50 °C/1 min, and 72 °C/1 min, then one cycle at 72 °C/10 min. The comC gene fragment produced was cloned into pUCm-T and sequenced from both ends with the original amplification primer with a Perkin-Elmer-ABI Prism 377 (Sangon Company, Shanghai, China).

The protein sequence of the putative CSP precursor was deduced based on the gene sequence of comC. The mature CSP sequence was determined as a cleavage product of its precursor protein by locating the cleavage site after a glycine-glycine consensus sequence commonly found in the leader sequence of Gram-positive peptide signal molecules (Havarstein et al., 1995). CSP peptide was synthesized using double-coupling cycles by standard 9-fluorenylmethoxycarbonyl (Fmoc) solid-phase synthesis methods (431A Peptide Synthesizer, Applied Biosciences or Apex396, Advanced Chemtech) as described previously (Eckert et al., 2003). Completed peptides were cleaved from the resin with 95% trifluoroacetic acid (TFA) with appropriate scavengers and purified by reverse-phase high-performance liquid chromatography (RP-HPLC; ACTA Purifier, Amersham) to 90–95%. Peptide molecular mass was determined by matrix-assisted laser desorption/ionization (MALDI) mass

### Identification of comC gene and synthesis of CSP of S. oligofermentans

The genomic DNA of S. oligofermentans was extracted and purified using the method of Marmur (1961) with slight modifications (Dong et al., 2000). The genomic sequences encompassing the entire arginine tRNA, comC and partial comD gene of some species in the genus Streptococcus were retrieved from GenBank and aligned using DNAMan. A pair of PCR primer SocomCF (5’-GCATANTCNTNGCAGAAA CC-3’) and SocomCR (5’-GTTCAATCNGCTGGGATCC-3’) was designed according to the homologous region of the comD and arginine tRNA sequence, respectively. The primers were synthesized by Sangon Company (Shanghai, China). The comC region of S. oligofermentans was amplified using its chromosome DNA as PCR template with a thermo-cycling machine (Thermolyhaid). The 25 µL PCR reaction mixture contained 100 ng DNA, 0.4 µM each pair of primer, 0.1 mM dNTP, 10 × PCR buffer and 0.1 U Taq polymerase (Takara Company, Dalian, China). PCR was performed at 95 °C/5 min, five cycles at 94 °C/30 s, 45 °C/1 min, and 72 °C/1 min, 30 cycles at 94 °C/30 s, 50 °C/1 min, and 72 °C/1 min, then one cycle at 72 °C/10 min. The comC gene fragment produced was cloned into pUCm-T and sequenced from both ends with the original amplification primer with a Perkin-Elmer-ABI Prism 377 (Sangon Company, Shanghai, China).

### Table 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristics</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>Strains</strong></td>
<td></td>
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<tr>
<td>E. coli DH5α</td>
<td>supE44 lacU169 (80 lacZM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1 luxS</td>
<td>Hanahan (1983); Surette et al. (1999)</td>
</tr>
<tr>
<td>S. oligofermentans AS 1.3089</td>
<td>Wild type, Sp&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Tong et al. (2003)</td>
</tr>
<tr>
<td>S. oligofermentans : Φ(idh–gfp)</td>
<td>AS 1.3089 :pTH1-ldh, gfp&lt;sup&gt;-&lt;/sup&gt;, Sp&lt;sup&gt;-&lt;/sup&gt;, gfp under idh promoter</td>
<td>This work</td>
</tr>
<tr>
<td>S. oligofermentans : Φ(idh–luc)</td>
<td>AS 1.3089 :pTH1-ldh, luc&lt;sup&gt;-&lt;/sup&gt;, Sp&lt;sup&gt;-&lt;/sup&gt;, luc under idh promoter</td>
<td>This work</td>
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<td><strong>Plasmids</strong></td>
<td></td>
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<tr>
<td>pCR2.1</td>
<td>Kan&lt;sup&gt;+&lt;/sup&gt; Amp&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>pUCm-T</td>
<td>Amp&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Shenergy</td>
</tr>
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<td>pFW5-gfp</td>
<td>Sp&lt;sup&gt;-&lt;/sup&gt; gfp</td>
<td>Kret et al. (2004)</td>
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<tr>
<td>pFW5-luc</td>
<td>Sp&lt;sup&gt;-&lt;/sup&gt; luc</td>
<td>Podbielski et al. (1999)</td>
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<td>pTH1-ldh</td>
<td>Sp&lt;sup&gt;-&lt;/sup&gt; gfp, gfp under S. oligofermentans idh promoter</td>
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<td>pTH2-ldh</td>
<td>Sp&lt;sup&gt;-&lt;/sup&gt; luc, luc under S. oligofermentans idh promoter</td>
<td>This work</td>
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spectrometry. The synthetic CSP was dissolved in sterile double-distilled water at a concentration of 10 mg mL\(^{-1}\) and stored at \(-20^\circ C\).

**Transformation of *S. oligofermentans* using synthetic CSP**

CSP concentrations varying between 0.2 and 1000 nM were tested for their effect on transformation of *S. oligofermentans*. Overnight culture of *S. oligofermentans* in BHI was 1:30 diluted into fresh BHI broth. After growing as static culture aerobically at 37°C for 30 min, transforming plasmids were added at a final concentration of 1 \(\mu\)g mL\(^{-1}\) together with CSP. A parallel assay without CSP was included as a control to determine the indigenous competence of *S. oligofermentans* at the tested time points during growth. After 2 h incubation, the cultures were plated on BHI agar containing 800 \(\mu\)g mL\(^{-1}\) spectinomycin and incubated at 37°C in a candle jar for 2 days to select transformants. The positive transformants were verified by PCR amplification of spectinomycin-resistant add9 gene and other enzymatic activities carried on the plasmids. The transformation efficiency was measured as the ratio of transformants vs. total number of viable cells per milliliter of culture.

**Construction of *ldh* green fluorescent protein (gfp) and *ldh* luciferase reporter strains of *S. oligofermentans***

The genomic DNA of *S. oligofermentans* was extracted as described previously. The sequences of lactate dehydrogenase (*ldh*) promoter region, including c. 1 kb of sequence upstream of the *ldh* start codon and partial sequence of *ldh* coding gene, from some species of oral streptococci were retrieved from GenBank and aligned using DNAman. A pair of primer SoldhF (5’-CNAANGCAACATTTNGCTCATG-3’) and SoldhR (5’-AANGCGTAAGANGAACCCTAC-3’) was designed according to the conserved region of the corresponding sequences and synthesized. The *ldh* promoter region was amplified from chromosome DNA of *S. oligofermentans* using PCR. The 25 \(\mu\)L PCR reaction mixture contained 100 ng DNA, 0.4 \(\mu\)M each of primer, 0.1 mM dNTP, 10 \(\times\) PCR buffer and 0.1 U Taq polymerase (Takara Company, Dalian, China). PCR was performed at 95°C/5 m in, five cycles at 94°C/30 s, 50°C/1 min and 72°C/1 min, 30 cycles at 94°C/30 s, 55°C/1 min and 72°C/1 min, then one cycle at 72°C/10 min. The *ldh* promoter fragment produced was cloned into pCR\(^\text{®}\) 2.1-Topo\(^\text{®}\) (Invitrogen) and verified by DNA sequencing. This fragment was subsequently cut out from pCR2.1 using BamHI and XhoI, gel purified, and ligated to the compatible sites on pFW5-luc (Podbielski et al., 1999) and pFW5-gfp (Kreth et al., 2004) vector using DNA ligase (Shernergy company, Shanghai, China). The ligation mixture was then transformed into *E. coli* DH5α. The correct plasmids were confirmed by restriction analysis and PCR.

Plasmids containing *ldh–gfp* (pTH1-ldh) and *ldh–luc* (pTH2-ldh) fusions were transformed into *S. oligofermentans* as described above. Transformants were selected on BHI agar containing 800 \(\mu\)g mL\(^{-1}\) spectinomycin and confirmed by PCR as well as by luciferase activity or green fluorescence emission.

**Visualization of *S. oligofermentans* *ldh–gfp* reporter in planktonic and biofilm cells**

For planktonic cells, *S. oligofermentans::F(*ldh–gfp)* was grown in BHI liquid for 24 h, then single cells were observed using fluorescent microscope. For biofilm assays, overnight culture of *S. oligofermentans::F(*ldh–gfp)* was 1:10 diluted into TH broth containing 0.5% sucrose and 1% glucose, and then inoculated into Lab-Tek\(^\text{®}\) II Chamber Slide\(^\text{TM}\) System (Nalge Nunc International, Naperville, IL). After the chambers were grown for 16 h in a candle jar, supernatants were removed from the chambers. Mature biofilms were exposed to air for 10 min in dark at room temperature, and then washed with PBS buffer. The biofilms were observed using a confocal laser scanning microscope (LEICA TCS SP2).

**Luciferase measurement of *S. oligofermentans* *ldh–luc* reporter**

Overnight culture of *S. oligofermentans::F(*ldh–luc)* was 1:30 diluted into BHI medium. The culture was sampled every 30 min from the early log to early stationary phase to measure luciferase activity and OD\(_{600}\) nm. Twenty-five microliters of 1 mM D-luciferin (Sigma) solution (suspended in 1 mM citrate buffer, pH 6.0) was added into 100 \(\mu\)L samples, and luciferase assays were performed essentially as described previously (Loimaranta et al., 1998) using a TD 20/20 luminometer (Turner system). OD\(_{600}\) nm was read with a 721 spectrophotometer (Shanghai analytical manufactory). All the measurements were done with duplicate samples, and all experiments were repeated at least three times.

**Results**

**Identification of *comC* gene and deduction of CSP sequence of *S. oligofermentans***

To identify the *comC* gene, we took advantage of the highly conserved genomic organization of the *comCDE* genes among members of the mitis group streptococci (Havarstein et al., 1996; Pestova et al., 1996). Sequences of the *comCDE* region from *S. pneumoniae*, *S. gordonii* and *S. mitis* were aligned, and degenerate primers were designed based on the highly conserved sequences. The forward primer was designed using a fragment of the conserved arginine tRNA sequence upstream of *comC* gene, and the reverse primer

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The text continues with further details on experiments and results.
was based on the homologous region of the \textit{comC} gene downstream of \textit{comD} gene of \textit{S. oligofermentans}. A \textit{comC}-containing PCR product of 1.0-kb fragment was sequenced and shown to have the highest homology to the ‘CSP’ gene of \textit{Streptococcus cristatus}. The deduced amino acid sequence of \textit{S. oligofermentans} CSP precursor is shown in Fig. 1. The predicted mature peptide is one amino acid longer (14 amino acids) than that of \textit{S. cristatus}, and the overall difference between the two CSPs is only five amino acids. In contrast, except for the C-terminal double lysine (KK) residues, the \textit{S. oligofermentans} CSP looks totally different than the CSPs of \textit{S. gordonii} and \textit{S. pneumoniae}.

**CSP-induced competence in \textit{S. oligofermentans}**

To determine whether the predicted CSP was indeed used as the signaling peptide for competence development in \textit{S. oligofermentans}, the 14-amino acid was chemically synthesized and used to induce competence in \textit{S. oligofermentans}. As shown in Fig. 2, similar to other CSPs, competence developed in response to CSP addition in a dose-dependent manner. It appeared that the threshold concentration of CSP was 10 nM, after which transformation frequency increased steadily with the concentration of CSP. The optimal concentration of CSP was found to be 500 nM. No transformants were obtained for \textit{S. oligofermentans} growing under the same conditions but without CSP addition, indicating that \textit{S. oligofermentans} did not display a natural competence under this growth condition and that transformation was completely induced by the addition of CSP.

**Construction of \textit{ldh–gfp} and \textit{ldh–luc} reporter strains with the synthetic CSP**

To construct reporter strains of \textit{S. oligofermentans} that could be monitored in situ planktonic and biofilm cultures, we used the \textit{ldh} gene promoter to drive the expression of the \textit{gfp} gene and the firefly luciferase gene (\textit{luc}), respectively. Ldh is a central metabolic enzyme that has been shown to be expressed constitutively in \textit{S. mutans} (Merritt \textit{et al.}, 2005). \textit{ldh–gfp} and \textit{ldh–luc} fusions have been used previously to monitor competition of \textit{S. mutans} with other oral streptococci (Kreth \textit{et al.}, 2005). To clone the \textit{ldh} promoter from \textit{S. oligofermentans}, \textit{ldh} genes from seven \textit{Streptococcus} species (five species in mitis group, two species in mutans group) were aligned, and the conserved regions were identified. A degenerate forward primer was designed from the conserved \textit{gyrA} gene region, which was located upstream but in an orientation opposite to the \textit{ldh} gene. A reverse primer was designed based on the homologous regions of the \textit{ldh} gene. A 1.1 kb PCR product was amplified using this pair of

**Fig. 1.** Sequence of the \textit{comC} gene in \textit{Streptococcus oligofermentans} and comparison with the precursor sequences of other CSPs. (a) sequence of the \textit{comC} gene in \textit{S. oligofermentans}. \textit{comC} region of \textit{S. oligofermentans} was amplified from genomic DNA and cloned into pUCm-T and sequenced from both ends. The GenBank accession number of this sequence is DQ640310. (b) Comparison of the deduced CSP precursor sequence with other CSPs. The cleavage site is indicated by the vertical arrow, after which is the mature CSP sequence. There were just two amino acids (underlined characters) different between the mature CSP sequences of \textit{S. oligofermentans} and \textit{Streptococcus cristatus}.

**Fig. 2.** Transformation frequency of \textit{Streptococcus oligofermentans} as a function of CSP concentration: Overnight culture of \textit{S. oligofermentans} was 1 : 30 diluted into fresh BHI broth, and the transforming plasmid was added to a final concentration of 1 \(\mu\)g \(\text{ml}^{-1}\). Synthetic CSP was also added at final concentrations varying between 0.2 and 1000 nM at the same time. The transformation efficiency was measured as the ratio of transformants vs. total number of viable cells per milliliter of culture. The results were expressed as the mean \pm standard error of three independent experiments.
primer. Sequence of this fragment showed 83% homology to gyrA and 91% homology to ldh of S. mutans at the nucleotide level (data not shown). This fragment was, therefore, assumed to contain the ldh promoter region and subsequently used to construct ldh–gfp and ldh–luc fusions (plasmid pTH1-ldh and pTH2-ldh plasmid, respectively). Plasmids pTH1-ldh and pTH2-ldh were transformed into S. oligofermentans with the assistance of CSP, and the plasmids were integrated into the chromosome through homologous recombination at the ldh promoter region, thus yielding the gfp and luc reporter strains S. oligofermentans::φ(ldh–gfp) and S. oligofermentans::φ(ldh–luc), respectively.

Expression of ldh–gfp reporter in planktonic and biofilm cells

To test whether ldh–gfp can be effectively expressed to make the cells visible under a fluorescent microscope, S. oligofermentans::φ(ldh–gfp) was grown in BHI broth for the planktonic culture and in TH broth supplemented with 1% glucose and 0.5% sucrose for the biofilm culture. After 24 h incubation in a candle jar, the planktonic cells were visualized under a fluorescent microscope, and the biofilm cells were examined using confocal laser scanning microscope. As shown in Fig. 3, both cells in planktonic and biofilm cultures displayed bright green fluorescence. In contrast, the parental strain did not show any fluorescence in either culture. These results indicate that the ldh–gfp reporter can be used to monitor the in situ growth of S. oligofermentans.

Correlation of ldh–luc expression with cell biomass

Expression of ldh–luc fusion has been used to estimate S. mutans cell numbers in mixed species cultures (Merritt et al., 2005). One important requirement for this purpose is that the expression of the reporter gene has to be consistent throughout the growth phases. Hence luciferase activities of the ldh–luc reporter strain were determined at various time points of the growth phase. As shown in Fig. 4, luciferase activity and culture turbidity (OD600 nm) followed the same trend until the culture entered into stationary phase. When the luciferase activity was normalized to OD600 nm, it showed nearly a flat line (data not shown). These results indicate that ldh–luc reporter strain could be used to determine the biomass of S. oligofermentans.

Discussion

The dental biofilm harbors over 700 bacterial species, among them S. mutans is considered as a primary pathogen for dental caries (Hamada & Slade, 1980). The mitis group streptococci have long been known to be the antagonists of S. mutans (Caufield et al., 2000; Becker et al., 2002). A recent study has shown that H2O2 production by members of the

Fig. 3. Expression of ldh–gfp gene fusions of Streptococcus oligofermentans in planktonic cells and in biofilm: S. oligofermentans::φ(ldh–gfp) and its parental strain were grown in BHI broth for the planktonic culture and in TH broth supplemented with 1% glucose and 0.5% sucrose for the biofilm culture. After 24 h incubation planktonic cells were visualized under a fluorescent microscope and biofilm cells were examined using confocal laser scanning microscope (CLSM). (a) and (b) phase-contrast picture of planktonic cells of S. oligofermentans::φ(ldh–gfp) and its parental strain, respectively; (c) and (d), phase-contrast picture of biofilm cells of S. oligofermentans::φ(ldh–gfp) and its parental strain, respectively; (e) and (f), fluorescent microscope picture of planktonic cells of S. oligofermentans::φ(ldh–gfp) and its parental strain, respectively; (g) and (h), CLSM picture of biofilm cells of S. oligofermentans::φ(ldh–gfp) and its parental strain, respectively. All pictures were taken with × 1000 magnification.
mitis group such as *Streptococcus sanguinis* and *S. gordonii* plays an important role in the competition and coexistence between the two streptococcal groups (Krith *et al.*, 2005). *Streptococcus oligofermentans* is a newly identified member of the mitis group. Preliminary studies have shown a strong correlation between *S. oligofermentans* and the caries-free status of the patients, suggesting a beneficial role of this bacterium in oral health. To gain a better understanding of the biology of this streptococcal species, in this study we developed a genetic system in *S. oligofermentans* that can be easily manipulated.

The *comC* gene was isolated by PCR using primers designed based on the conserved sequences in the *comCDE* locus of the mitis group streptococci. The deduced mature peptide sequence of ComC (CSP) showed the highest similarity with the CSP of *S. crista* than with any other members of the mitis group. This suggests that *S. oligofermentans* may be genetically much closer to *S. crista*, while further study is needed to determine whether there is a CSP cross-recognition between the two species.

Using chemically synthesized CSP based on the deduced sequence of the mature peptide from ComC, the competence ability of *S. oligofermentans* was explored. *Streptococcus oligofermentans* responded to CSP in a dose-dependent manner just like other members of the mitis group such as *S. pneumoniae* did, suggesting that it possessed a complete set of functional competence genes. However, unlike *S. pneumoniae*, competence did not develop naturally under the experimental conditions that we used, as shown by the absence of transformants in the absence of exogenous CSP. It is highly likely that competence does develop naturally but at an appropriate growth condition has yet to be discovered.

Using the synthetic CSP to induce transformation in *S. oligofermentans*, we constructed two reporter systems. The *ldh–gfp* reporter showed high level expression both in planktonic and biofilm cells, indicating the utility of this reporter strain in future studies such as competition between *S. oligofermentans* and *S. mutans* in planktonic and biofilm conditions. On the other hand, the constitutive expression pattern of the *ldh–luc* reporter strain would provide a means to quantify *S. oligofermentans* biomass in mixed cultures. We anticipate that development of the genetic system and construction of the reporter strains of *S. oligofermentans* will tremendously facilitate the understanding of the biology of this potentially beneficial bacterium.

### Acknowledgements

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