Construction of Transcriptional lacZ Reporter Gene Strains to Monitor the Activity of a Peptide-Mediated Two-Component Transduction System in Streptococcus mutans. Alan Salman, Song F. Lee and Yung-Hua Li*.
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ABSTRACT

Two-component transduction systems employ a histidine kinase-sensing histidine phosphotransfer protein (HPr) signaling cascade to regulate gene expression. HPr binds to autocatalytic histidine residues to activate a conserved aspartate protease domain, which catalyzes the dephosphorylation of the phosphoamide group, thereby deactivating the signal. This study describes the construction of transcriptional lacZ reporter gene strains to monitor the activity of a signaling peptide-mediated two-component transduction system in S. mutans.

INTRODUCTION

Maturation of signaling peptides is one of the major routes that regulate cell division and metabolism in bacteria. Peptide signal transduction often employs a two-component system that involves the interaction of a histidine kinase (HPr) and a response regulator. The HPr senses the signal and functions as a phosphorelay, transferring the phosphate from the histidine residue to the histidine of the response regulator. The receptor, in turn, regulates the expression of target genes.

OBJECTIVE

• To construct transcriptional lacZ reporter gene strains to monitor the activity of a signaling peptide-mediated two-component transduction system in S. mutans.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media: Bacterial strains and plasmids used in this study are listed in Table 1. S. mutans strain UA159 was grown in Todd-Hewitt Yeast Extract (THYE) medium, while the strains derived from this microorganism (S. mutans) were grown in THYE plus a protein supplement. pUC18 vector was used to transform S. mutans strain UA159.

Transformation into S. mutans strains: The integrative plasmid SMwt-PcomDE-lacZ was transformed into both S. mutans UA159 and the ctrP deficient mutant to create two lacZ reporter strains as described in Fig. 1. We also transformed plasmid SMwt-PcomDE into these two strains to monitor background levels (Fig. 4).

RESULTS

1. We have successfully constructed an E. coli-S. mutans shuttle vector harboring a lacZ transcriptional reporter fused with the promoter of comCDE, allowing the monitoring of two-component transduction system that senses and responds to quorum-sensing signaling peptides from S. mutans.

2. This lacZ reporter construct has been transformed into both S. mutans wild type UA159 and the ComC mutant strain. Both reporter strains have been confirmed to express reduced β-galactosidase activity in response to signaling peptides.

ACKNOWLEDGMENTS

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REFERENCES


Table 1. Bacterial Strains And Plasmids Used In This Study

<table>
<thead>
<tr>
<th>Strain or Plasmid</th>
<th>Relevant Characteristics</th>
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<td>UA159</td>
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<td>SMwt-PcomDE-lacZ</td>
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Figure 1. Diagram of transcriptional reporter constructs for detecting quorum-sensing signaling peptide activity in S. mutans. The reporter strain was constructed by integrating the transcriptional lacZ reporter into the S. mutans chromosome or plasmid. The reporter strain was transformed with the plasmid carrying the transcriptional lacZ reporter and the S. mutans tester strain was grown in THYE medium containing inducer. The level of β-galactosidase activity was measured using the o-nitrophenol-borate method.

Figure 2. Diagram of the two-component signaling pathway in S. mutans. The signaling peptide (ComB) is sensed by the histidine kinase (ComA), which phosphorylates the response regulator (ComD). The phosphorylated response regulator then binds to the target gene promoter (lacZ) and activates transcription, resulting in the production of β-galactosidase activity.

Figure 3. Graph showing the activity of the lacZ reporter in S. mutans strain UA159. The activity was measured using the o-nitrophenol-borate method.

Figure 4. Graph showing the activity of the lacZ reporter in S. mutans strain UA159 with and without the ComC signaling protein. The activity was measured using the o-nitrophenol-borate method.