Isolation and Characterization of

Pseudobutyrivibrio ruminis Gene

Promoters

Tobias Schoep


This thesis is presented for the degree of

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I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

__________________________

Tobias Delavilla Schoep
A family of *E. coli - P. ruminis* shuttle-plasmids was constructed to allow the isolation and characterization of gene promoters from the rumen bacterium *P. ruminis*. The promoter rescue plasmid pBK was used to isolate a total of 4 genomic DNA fragments that promoted transcription in *P. ruminis* strains 0/10. These promoters, and an additional promoter, previously isolated from *P. ruminis* strain OR38 (Schoep, 1999), were identified by their ability to initiate expression of a promoterless *ermAM* gene in *P. ruminis*. Within 4 of the fragments, a total of 5 transcription start sites were identified in *P. ruminis* using a novel, fluorescent-primer extension analysis protocol. Comparison of promoters isolated in this and previous studies revealed a strong consensus RNA polymerase DNA-binding motif, including the well characterized –35 and –10 elements. Consensus sequences established for these elements were: TTgacA and A[IAAT][A], respectively, where bold upper-case font, regular upper-case, and lower-case fonts represent conservation in 100%, 80%, and 70% of promoters respectively. The –10 and –35 motifs were interspaced by 16 – 18 nt. Among the newly identified promoters, the consensus for the –10 element was extended one nucleotide upstream and downstream of the standard hexamer (boxed). These motifs were similar to those recognized by eubacterial RNA polymerase containing the σ^70^-like factor. Promoters also contained possible UP elements, and were significantly more curved than protein-coding regions. Additional plasmid vectors were constructed, to allow the use of both the quantitative SYBR green real time PCR and β-glucuronidase assays, to examine 4 promoters in depth. This showed a wide range of promoter strengths within the group. However, no correlation was found between the composition and context of elements within *P. ruminis* promoters, and promoter strength. A mutation within the –35 element of one promoter revealed that promoter strength, and the choice of transcription start site were both sensitive to single nucleotide changes.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Expansion</th>
</tr>
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<tr>
<td>ABI</td>
<td>Applied Biosystems Inc.</td>
</tr>
<tr>
<td>AMV</td>
<td>Avian myeloblastosis virus</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine-5'-triphosphate</td>
</tr>
<tr>
<td>AR</td>
<td>analytical reagent</td>
</tr>
<tr>
<td>b</td>
<td>bases</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>DEPC</td>
<td>diethyl pyrocarbonate</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic Acid</td>
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<td>deoxynucleotides</td>
</tr>
<tr>
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<td>double stranded</td>
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<td>IPTG</td>
<td>isopropyl-β-D- thiogalactopyranoside</td>
</tr>
<tr>
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</tr>
<tr>
<td>MMLV</td>
<td>Moloney murine leukemia virus</td>
</tr>
<tr>
<td>OD</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>pers. comm.</td>
<td>personal communication</td>
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<td>pNPG</td>
<td>p-Nitrophenyl β-D-Glucuronide</td>
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<td>ribonuclease</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
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<td>TAE</td>
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<td>Tris-borate EDTA buffer</td>
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<td>5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside</td>
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