Shedding light on betL*: pPL2-lux mediated real-time analysis of betL* expression in Listeria monocytogenes

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ABSTRACT

We propose a mechanism of action for the betL* mutation which is based on DNA topology. Removing a single thymine residue from the betL* promoter's −10 and −35 spacer results in a 'twist'-mediated activation of transcription which accounts for the osmotolerance phenotype observed for strains expressing betL*. 

KEYWORDS

betL*; lux; Listeria; osmotolerance

Work in our labs, and others, has shown osmo-regulatory control in the intracellular foodborne bacterial pathogen Listeria monocytogenes to be elaborately orchestrated. This is particularly true of BetL, a key component of the listerial osmotolerance response and a versatile stress tolerance locus. One of the primary respondents in the secondary response to osmotic up-shock, betL (and its encoded membrane protein, BetL) is regulated at the transcriptional, translational and post-translational levels. Indeed, we have shown that betL is controlled by at least 2 putative promoter elements: σA and σB. While the latter is a global stress regulator and a key component of the pathogen's gastrointestinal phase of infection; the former is more usually associated with general housekeeping activities. However, we recently described a single point mutation (deletion of a thymine residue) in the putative betL σA promoter which dramatically improves the pathogen's osmotolerance profile; suggesting a previously unreported role for this promoter in the listerial osmoregulatory response. Herein, we describe the use of pPL2lux, a luciferase-based reporter system, to monitor the transcriptional profile of betL in real-time, thereby enabling us to pinpoint when and to what extent the mutation influences transcription, both in the presence and absence of salt stress.

A derivative of the listerial integration vector pPL2, which exhibits site-specific, single-copy integration into the L. monocytogenes chromosome, pPL2lux harbours a synthetic luxABCDE operon encoding both the substrate and enzyme required to produce measurable quantities of light. Furthermore, given that the luxABCDE operon was derived from pSB2025 with the introduction of a blunt-end SwaI restriction site overlapping the ATG start codon of luxA; cloning between SalI-SwaI facilitates exact translational fusions to the lux reporter, mimicking transcription and translation initiation as it occurs at the native chromosomal location of the promoter under investigation. Using this system, the level of light emitted in real-time is directly proportional to the level of transcription.

In the current study, the betL and betL* promoter elements (PbetL and PbetL*), were PCR amplified from previously constructed plasmids, pRS3 and pRS2 respectively, using KOD polymerase (Merck) with primers betLPR (5'-CAATGTCGACCCACGCT-CACCGGCTCCAG-3' SalI restriction site underlined) and betLPR (5'-CAATACATCATTCCCTTT
ATTTTC-3'). The resulting ~0.3 kb PCR products contained the regulatory regions immediately upstream of betL and betL*, respectively. The P<sub>betL</sub> and P<sub>betL*</sub> amplicons were digested with SalI and cloned into SwaI-SalI-digested pPL2lux, yielding pPL2lux-P<sub>betL</sub> and pPL2lux-P<sub>betL*</sub>, respectively. Subsequently, pPL2lux (negative control), pPL2lux-P<sub>betL</sub>, and pPL2lux-P<sub>betL*</sub> were transformed into L. monocytogenes LO28, and candidate integrants were checked for site-specific integration by PCR, using primers PL95 (5'-ACATAATCAGTCCAAAGTAGATGC-3') and PL102 (5'-TATCAGCCTAACCCAAACCTTC C-3'). From each transformation, several colonies with the correct genotype were selected for phenotypic confirmation by bioluminescent imaging, using the Xenogen IVIS 100 system (Xenogen, Alameda, CA).

Overnight cultures of the confirmed strains (LO28::pPL2lux, LO28::pPL2lux-P<sub>betL</sub>, and LO28::pPL2lux-P<sub>betL*</sub>) were diluted 1:50 in Tryptone Soya Broth (TSB). Growth and bioluminescence were monitored simultaneously from lag to stationary phase, in the presence and absence of added salt (Fig. 1).

While previously no significant differences in transcript levels were observed between betL* and the wild-type gene in the absence of salt stress (using an RT-PCR based approach<sup>10</sup>) this was not the case in the current study. Indeed, expression levels from LO28::pPL2lux-P<sub>betL</sub> appeared markedly higher than that of LO28::pPL2lux-P<sub>betL*</sub>, particularly during the lag and log phases of growth. Furthermore, while LO28::pPL2lux-P<sub>betL</sub> expression levels began to decrease in mid log phase, LO28::pPL2lux-P<sub>betL*</sub> expression remained steady until late log phase (Fig. 1A). Interestingly, for both cultures, expression rebounded in stationary phase, suggesting the involvement of a promoter other than the putative σ<sup>54</sup>; possibly σ<sup>B</sup>, or an as yet unidentified stationary phase specific promoter.

In the presence of 4% added NaCl (the optimal salt concentration for BetL activity<sup>9</sup>), similar, albeit more dramatic trends were observed. Expression levels for LO28::pPL2lux-P<sub>betL</sub> were significantly higher than those of LO28::pPL2lux-P<sub>betL*</sub> from the outset and remained so until early stationary phase. Indeed, while LO28::pPL2lux-P<sub>betL</sub> expression levels were no longer detectable after 13 hours, LO28::pPL2lux-P<sub>betL*</sub> expression remained steady up to hour 16, only gradually decreasing to hour 24. Resurgence in expression in stationary phase was again observed for both cultures in the presence of 4% NaCl, further suggesting the involvement of an alternative stationary phase promoter.

Finally, based on the above observations, we propose a mechanism of action which is based on DNA topology. If, as we suspect, the betL σ<sup>54</sup>-like promoter belongs to a class of DNA twist-sensitive promoters<sup>21</sup>; removing a single thymine residue from the promoter’s –10 and –35 spacer region would, as we have observed, boost transcription of betL even in the absence of salt stress. Furthermore, given previously well documented links between osmolarity and DNA supercoiling<sup>22-25</sup>; the addition of salt is likely to lead to further activation of betL expression. This exogenous
‘twist’-mediated activation, in the presence of NaCl, boosts already elevated transcript levels; resulting in the dramatic osmotolerance phenotype observed for strains expressing betL*.

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No potential conflicts of interest were disclosed.

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