Genome-wide mapping of transcription start sites yields novel insights into the primary transcriptome of Pseudomonas putida

The environmental bacterium *Pseudomonas putida* is an organism endowed with a versatile metabolism and stress tolerance traits that are desirable in an efficient production organism. In this work, differential RNA sequencing was used to investigate the primary transcriptome and RNA regulatory elements of *P. putida* strain KT2440. A total of 7937 putative transcription start sites (TSSs) were identified, where over two-thirds were located either on the opposite strand or internal to annotated genes. For TSSs associated with mRNAs, sequence analysis revealed a clear Shine–Dalgarno sequence but a lack of conserved overrepresented promoter motifs. These TSSs defined approximately 50 leaderless transcripts and an abundance of mRNAs with long leader regions of which 18 contain RNA regulatory elements from the Rfam database. The thiamine pyrophosphate riboswitch upstream of the thiC gene was examined using an *in vivo* assay with GFP-fusion vectors and shown to function via a translational repression mechanism. Furthermore, 56 novel intergenic small RNAs and 8 putative actuation transcripts were detected, as well as 8 novel open reading frames (ORFs). This study illustrates how global mapping of TSSs can yield novel insights into the transcriptional features and RNA output of bacterial genomes.

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