Original Research ARTICLE

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The Novel Anaerobiosis-Responsive Overlapping Gene *ano* Is Overlapping Antisense to the Annotated Gene ECs2385 of *Escherichia coli* O157:H7 Sakai

Sarah M. Hücker[1], Sonja Vanderhaeghen[1], Isabel Abellan-Schneyder[1], Siegfried Scherer[1,2] and Klaus Neuhaus[1,3*]

- [1]Chair for Microbial Ecology, Technical University of Munich, Freising, Germany
- [2]Institute for Food & Health, Technical University of Munich, Freising, Germany
- [3]Core Facility Microbiome/NGS, Institute for Food & Health, Technical University of Munich, Freising, Germany

Current notion presumes that only one protein is encoded at a given bacterial genetic locus. However, transcription and translation of an overlapping open reading frame (ORF) of 186 bp length were discovered by RNAseq and RIBOseq experiments. This ORF is almost completely embedded in the annotated L,D-transpeptidase gene ECs2385 of *Escherichia coli* O157:H7 Sakai in the antisense reading frame -3. The ORF is transcribed as part of a bicistronic mRNA, which includes the annotated upstream gene ECs2384, encoding a murein lipoprotein. The transcriptional start site of the operon resides 38 bp upstream of the ECs2384 start codon and is driven by a predicted σ^{70} promoter, which is constitutively active under different growth conditions. The bicistronic operon contains a ρ-independent terminator just upstream of the novel gene, significantly decreasing its transcription. The novel gene can be stably expressed as an EGFP-fusion protein and a translationally arrested mutant of *ano*, unable to produce the protein, shows a growth advantage in competitive growth experiments compared to the wild type under anaerobiosis. Therefore, the novel antisense overlapping gene is named *ano* (anaerobiosis responsive overlapping gene). A phylostratigraphic analysis indicates that *ano* originated very recently *de novo* by overprinting after the *Escherichia/Shigella* clade separated from other enterobacteria. Therefore, *ano* is one of the very rare cases of overlapping genes known in the genus *Escherichia*.