Transposon-insertion sequencing screens unveil requirements for EHEC growth and intestinal colonization

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Enterohemorrhagic Escherichia coli O157:H7 (EHEC) is an important food-borne pathogen that colonizes the colon. Transposon-insertion sequencing (TIS) was used to identify genes required for EHEC and E. coli K-12 growth in vitro and for EHEC growth in vivo in the infant rabbit colon. Surprisingly, many conserved loci contribute to EHEC's but not to K-12's growth in vitro. There was a restrictive bottleneck for EHEC colonization of the rabbit colon, which complicated identification of EHEC genes facilitating growth in vivo. Both a refined version of an existing analytic framework as well as PCA-based analysis were used to compensate for the effects of the infection bottleneck. These analyses confirmed that the EHEC LEE-encoded type III secretion apparatus is required for growth in vivo and revealed that only a few effectors are critical for in vivo fitness. Over 200 mutants not previously associated with EHEC survival/growth in vivo also appeared attenuated in vivo, and a subset of these putative in vivo fitness factors were validated. Some were found to contribute to efficient type-three secretion while others, including tatABC, oxyR, envC, acrAB, and cvpA, promote EHEC resistance to host-derived stresses. cvpA is also required for intestinal growth of several other enteric pathogens, and proved to be required for EHEC, Vibrio cholerae and Vibrio parahaemolyticus resistance to the bile salt deoxycholate, highlighting the important role of this

previously uncharacterized protein in pathogen survival. Collectively, our findings provide a comprehensive framework for understanding EHEC growth in the intestine. © 2019 Warr et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.