A First Look at the Essential Genes of *Pseudomonas protegens*

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**ABSTRACT** Transposon insertion sequencing is a useful tool to identify the genes that are essential for a bacterial species to grow and divide effectively. In this issue of *Journal of Bacteriology*, Fabian et al. present the first set of transposon insertion sequencing data highlighting the genes essential to the plant-commensal species *Pseudomonas protegens* strain Pf-5 and describe comparative analyses with other pseudomonads (B. K. Fabian, C. Foster, A. J. Asher, L. D. Elbourne, et al., J Bacteriol 203:e00432-20, 2021, [https://doi.org/10.1128/JB.00432-20](https://doi.org/10.1128/JB.00432-20)).

**KEYWORDS** *Pseudomonas protegens*, transposon insertion sequencing, essential genes

In this issue, Fabian et al. (1) describe transposon insertion sequencing on the plant-commensal biocontrol bacterium *Pseudomonas protegens* strain Pf-5. By first creating a pool of ~256,000 unique transposon insertion mutants on rich LB medium, they sequenced these mutants and determined the genes where transposon insertions were permissible (nonessential genes) versus genes that were devoid of insertions (essential genes). They found 446 essential genes in this rich medium, accounting for 7.6% of the genome, which is comparable to the proportion of essential genes in other bacteria. However, comparison of these essential genes with those from three other pseudomonads (*P. simiae* WCS417, *P. aeruginosa* PA14, and *P. syringae* pv. *syringae* B728a) shows that the number of overlapping essential genes is only 235. This demonstrates the vast differences in gene essentiality between bacterial species, even within the same genus.

This important tool has largely been used for pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *P. aeruginosa*, but an increasing number of complete genome sequences are now available for relatively understudied species. This allows for the investigation of essential genes in more, and potentially all, sequenced bacterial species. While a genetic clean deletion of a gene is considered the gold standard in determining gene essentiality of a given strain, the process of making an arrayed library of individual deletions is extremely time-consuming and has been performed for only a small number of species, most notably *E. coli* strain K-12 from the Keio collection (2). Even then, a small number of truly essential genes could be duplicated during the construction process, allowing for inadvertent labeling of the gene as nonessential (3). Additionally, the added manipulations of a clean deletion library increase the probability of other compensatory mutations occurring. A comparison between the Keio collection and transposon mutagenesis experiments for *E. coli* strain K-12 has highlighted differences in approaches, although there is an overall commonality in the results between methods (4). Transposon insertion sequencing has an obvious advantage of speed and cost, but also, if carefully designed, it can be selected on any growth medium.

Two important limitations that Fabian et al. address in this issue are drawing broad conclusions of gene essentiality across a species from data from a single strain and also from the selection of rich LB medium, which is not necessarily representative of the native niche of the species (in this case, plants). First, the authors wisely analyzed the core genome of *P. protegens*, clustering genes that were found in all sequenced
strains, versus the accessory genome, genes that are found in only the strain studied. They found that in fact 397 of the 446 essential genes were from the core genome, with the remainder being part of the accessory genome. This raises interesting evolutionary questions, and further studies are warranted for deciphering essential genes found in the accessory genome. Second, the authors highlight the limitation of medium. LB medium is the most popular choice for growth of pseudomonads but does not fully represent the various environments that these bacteria survive and proliferate in. Future essential gene studies of plant-mimetic media for P. protegens would highlight interesting differences, similar to the extensive media and strain studies performed with P. aeruginosa (5–7). Nonetheless, Fabian et al. performed detailed comparisons between P. aeruginosa and P. protegens in LB medium and found an 80% overlap in essential genes between species, with the majority of these genes involved in basic cellular functions such as replication, translation, and cell envelope biogenesis.

A final caveat is the analysis challenges of transposon insertion sequencing data. While most cases of essential genes versus nonessential genes are obvious when performing such analysis, there is always a subset of genes without a clear divide between the two categories. Further, statistical analysis methods vary greatly; thus, a universal method would be useful as additional data are collected to make comparisons between studies and of different species. The current work is a step toward gaining a further understanding of plant-associated bacteria. Transposon insertion sequencing serves as an important tool in gaining additional insights on gene essentiality across the bacterial domain and specifically in relatively understudied organisms. Knowledge of essential genes is a crucial step for our own exploitation, for example, in developing broad-spectrum or narrow-spectrum antibiotics, which of course target essential genes.

REFERENCES