

VNTR-based typing of *Burkholderia cenocepacia*

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Abstract:

Conventional bacteriological genotyping methods, such as pulsed-field-gel-electrophoresis (PFGE), randomly amplified polymorphic DNA (RAPD) typing, and even multilocus sequence typing (MLST), discriminate variation at the level of strains but often miss variation arising within strains, such as that within an epidemic. The *B. cenocepacia* strain J2315¹ genome features numerous regions of repeated sequence that in principle should be prone to high rates of mutation. These regions, typically known as variable-number tandem repeats (VNTRs), are distributed evenly across the three chromosomes, are relatively rare on the plasmid, but tend to form statistically significant clusters. We identified more than 40 candidate regions that, because of their relatively large size (>50bp) and repeat number (>6), were amenable to typing by PCR and agarose gel electrophoresis; of these candidates, we have evaluated more than 20. Because PCR primers were designed using the J2315 genome sequence, some target regions were amplified only in the ET12 lineage, which suggests loci unique to this lineage. In contrast, other fragments were amplified from multiple species within the *B. cepacia* complex; as predicted, these were highly variable in size. We focused on those regions that could be amplified in nearly all *B. cenocepacia* tested, and in particular, among a group of isolates of strain PHDC that are relatively invariant according to other typing methods. Several VNTRs illustrated fine-scale variation within the PHDC clonal complex, and when combined these data revealed unique genotypes. We used this approach to quantify genetic variation arising within a limited geographic region where CF patients were frequently and persistently infected by a common PHDC strain over two decades.

1. These sequence data were produced by the *Burkholderia cenocepacia* Sequencing Group at the Sanger Institute and can be obtained from <ftp://ftp.sanger.ac.uk/pub/pathogens/bc/>.