GENOME WATCH

Bringing *Treponema* into the spotlight

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This month's Genome Watch highlights how culture-independent selective enrichment approaches coupled to whole-genome sequencing enable the analysis of unculturable microorganisms.

High-throughput sequencing approaches have proved invaluable in identifying genome variation, population dynamics and the virulence determinants of many microorganisms, including important human pathogens. This technology necessitates that the target organisms can be cultured under laboratory conditions; however, this is not always possible. For example, although the sexually transmitted bacterium *Treponema pallidum* subsp. *pallidum*, which is the causative agent of syphilis, and its related subspecies can be propagated in rabbits, they cannot be cultured *in vitro*. The reported genome sequence of *T. pallidum* subsp. *pallidum* by Fraser *et al.*[1] was a great advance in the field; however, until recently, population-level studies have not been possible owing to the difficulty in culturing pathogenic spirochetes.

Two recent studies have overcome this problem by coupling whole-genome sequencing (WGS) to a culture-independent SureSelect^{XT} enrichment approach. In the first study, Pinto *et al.*[2] used RNA oligonucleotides that were designed on the basis of available *T. pallidum* subsp. *pallidum* reference genomes as bait, and they were able to obtain the nearly complete sequences (>99%) of 24 strains from samples that were collected from infected individuals from Portugal. Their results revealed that the Portuguese strains were part of the SS14 sublineage, which is known to be widespread globally and highly homogeneous. Most of the detected variants were restricted to a set of genes, including the two copies of the 23S gene. Mutations in the 23S gene have been associated with resistance to azithromycin, which is a second-line antibiotic for the treatment of syphilis but a first-line antibiotic for the treatment of other sexually transmitted infections. Interestingly, their data suggest that extensive intra-patient subpopulation diversity is a potential mechanism to evade the host immune system and thus promote survival, dissemination and persistence. This variation was found to occur mainly in specific genes and functional categories, including membrane-associated, chemotaxis-associated and flagella-associated genes, which suggests a possible role in pathogenesis. The level of in-depth intra-patient sequencing that was achieved enabled the identification of mechanisms that are used by *T. pallidum* subsp. *pallidum* to gain phenotypic diversity, such as phase variation of homopolymeric tracts and hyper-mutability of the antigen-coding *tprK* gene. This is in agreement with the notion that ongoing adaptive diversification during human infection promotes pathogenesis and persistence.

In the second study, Arora *et al.*[3] applied a DNA capture microarray to analyse both rabbit-propagated samples and clinical samples that were collected from different countries. They were able to obtain a large proportion of the genomes (>80%) for 28 strains of *T. pallidum* subsp. *pallidum*, as well as *T. pallidum* subsp. *pertenue* and *T. pallidum* subsp. *endemicum*, which are the causative agents of yaws and bejel diseases, respectively. Phylogenetic analysis revealed a distinct clade for each subspecies, with more than 1,000 SNPs identified between the isolates from *T. pallidum* subsp. *pallidum* and the other two subspecies. Two sublineages were identified in the syphilis clade, and the SS14 sublineage, although being geographically more widespread, was less diverse than the Nichols sublineage. Moreover, the amount of genomic data obtained in the study was sufficient to infer the emergence of modern *T. pallidum* subsp. *pallidum*, and thus, modern syphilis, using Bayesian methods. Interestingly, the results indicated an emergence of this species after the fifteenth century, coinciding with the early modern era, and a more recent expansion of the SS14 epidemic sublineage in the twentieth century. Consistent with previous reports and with Pinto *et al.*, this study also confirmed the abundance of azithromycin-resistant strains in this sublineage.

The development of WGS has rapidly advanced every field in microbiology; however, the inability to sequence uncultured bacteria has hampered the study of many microorganisms, including important human pathogens. Nonetheless, culture-independent selective enrichment approaches are now being applied to recover the genome sequences of microorganisms that are notoriously difficult to culture. Results from such studies could increase our understanding of global genomic epidemiology, genome variation and population dynamics of uncultivable microorganisms and even help guide the development of novel treatments.

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doi:10.1038/nrmicro.2017.23
Published online 13 Mar 2017

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Competing interests statement
The authors declare no competing interests.