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Note

Whole-genome mapping for high-resolution genotyping of *Pseudomonas aeruginosa*

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ABSTRACT

A variety of molecular typing techniques have been developed to investigate the clonal relationship among bacterial isolates, including those associated with nosocomial infections. In this study, the authors evaluated whole-genome mapping as a tool to investigate the genetic relatedness between *Pseudomonas aeruginosa* isolates, including metallo beta-lactamase-positive outbreak isolates.

Pseudomonas aeruginosa is an opportunistic pathogen that is a major cause of nosocomial infections, it being associated with significant morbidity and mortality in critically ill and immune-compromised patients (Lyczak et al., 2000). These infections are often hard to treat due to the intrinsic and acquired resistance of *P. aeruginosa* against many different types of antibiotics (Breidenstein et al., 2011; Maltezou, 2009). It is therefore extremely important to apply timely and correct infection prevention and control measures in hospitals and care centres in order to monitor and prevent the spread of these highly resistant microorganisms. A variety of molecular genotyping techniques have been developed to investigate the genetic relatedness among bacterial pathogens, generating information useful in helping trace, monitor, and prevent the source of nosocomial outbreaks of infection. A relatively new typing technique that has become available is whole-genome mapping (WGM), which generates high-resolution, ordered, restriction maps that span the entire genome of bacteria (Miller, 2013). Further, WGM has been previously utilized to investigate closely related isolates of *Escherichia coli* (Chen et al., 2006; Kotewicz et al., 2008) and *Staphylococcus aureus* (Bosch et al., 2013; Clarridge et al., 2013), though no information has been published regarding the use of WGM for genotyping *P. aeruginosa* isolates. Therefore, a study was conducted in order to evaluate the use of WGM to discriminate between clinically relevant *P. aeruginosa* isolates, including nosocomial outbreak-associated isolates.

A total of 32 *P. aeruginosa* isolates were selected for this study. Twenty of these isolates were clinical isolates collected between 2009 and 2011, comprising 19 isolates from The Netherlands (P2–P38) and a single isolate from Canada (P17). Eight of these isolates had been previously found to be metallo beta-lactamase (MBL)-positive by PCR (Pitout et al., 2005). The corresponding whole-genome maps were prepared by OpGen, Inc. (Gaithersburg, Maryland, USA) using the *Bam*HI restriction enzyme, as previously described (Latreille et al., 2007). An extra twelve whole-genome maps were generated from publicly available whole-genome sequences of the following *P. aeruginosa* strains: 19BR (GenBank: AFXJ01000001), B136-33 (CP004061), c7747m (CP006728), DK2 (CP003149), LESB58 (FM209186), M18 (CP002496), MTB-1 (CP006853), NCGM2.S1 (AP012280), PA7 (CP000744), PAO1 (AE004091), RP73 (CP006245), and UCBPP-PA14 (CP000438). This is a convenient method for increasing the number of WGM isolate maps available for analysis and helps place newly WGM mapped bacterial isolates in the context of global isolates and outbreaks.

The resulting *in vitro* and *in silico P. aeruginosa* whole-genome maps were aligned and compared using BioNumerics v7 software (Applied Maths, Sint-Martens-Latem, Belgium). Restriction fragments smaller than 3000 bp were excluded from the analysis and a relative tolerance of 15%, combined with an absolute tolerance of 3000 bp, was used to compensate for WGM resolution. All dendrograms were generated using UPGMA with a map distance of 5% (>95% similarity) as a cut-off point to define a WGM cluster, as reported previously (Bosch et al.,

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2013). Importantly, the BioNumerics v7 software allows two different approaches to be made when comparing whole-genome map similarities: 1) alignment-based comparison and 2) pattern-based comparison. The alignment-based approach uses a size tolerance algorithm for pairwise comparison of the WGM fragments, whereas the pattern-based approach takes into account a neighbourhood of fragments, matching within a given size tolerance. To explore both methods, we applied them to our set of 32 whole-genome maps. As shown in Fig. 1, at the 95% similarity

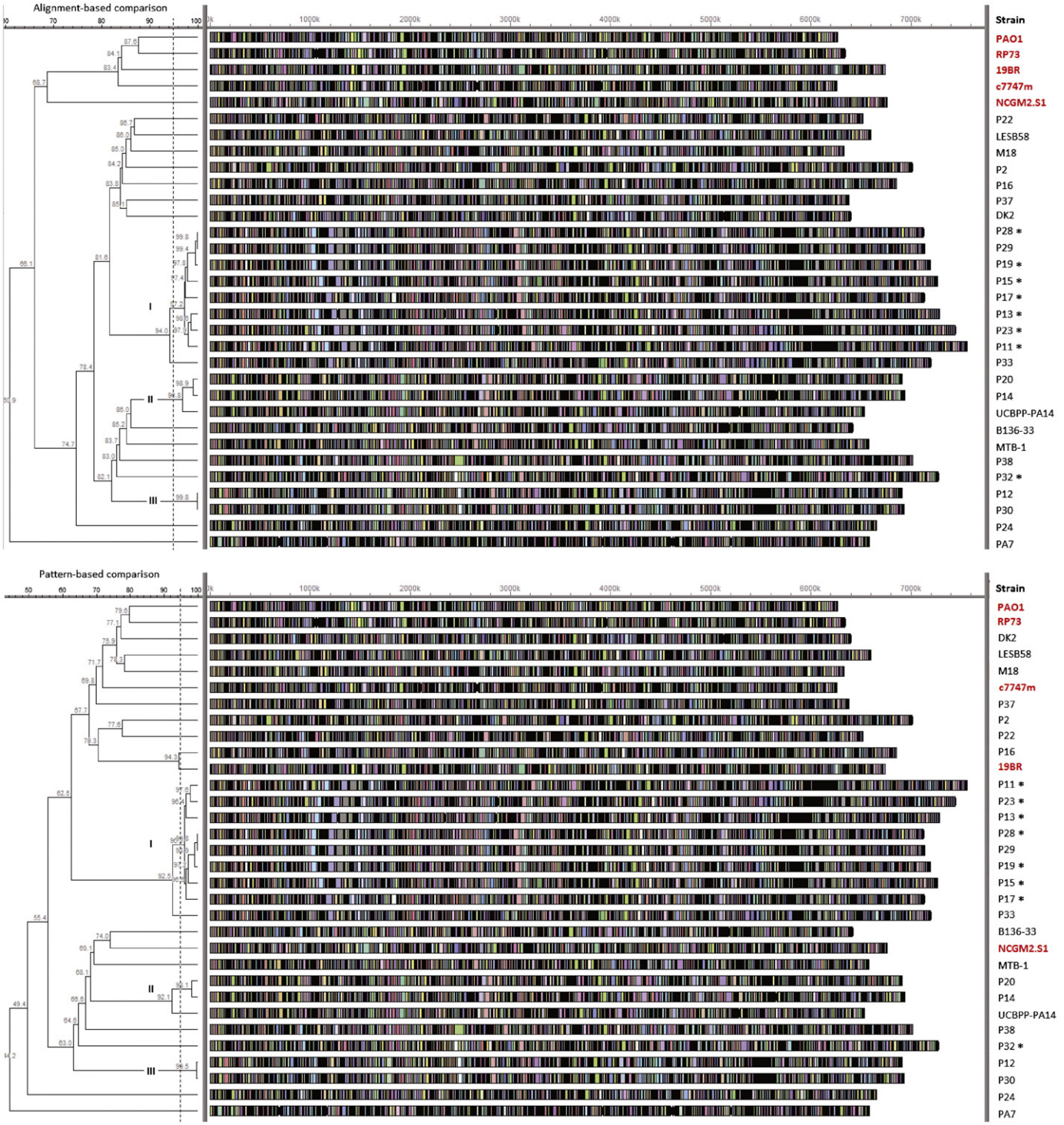

Fig. 1. Differences observed between alignment-based and pattern-based algorithms when comparing WGM data derived from 32 *P. aeruginosa* strains. The alignment-based comparison (top) shows different clustering results for the *in silico* digested isolates PAO1, RP73, 19BR, c7747m and NCGM2.S1 (indicated in red) compared to the pattern-based comparison (bottom). Close inspection of the whole-genome maps reveals that chromosomal inversions among these strains are causing the clustering differences. Three clusters (I–III) can be identified using a similarity cut-off of 95%. The clustering of groups I–III isolates is similar using both alignment-based and pattern-based algorithms. * = metallo beta-lactamase PCR-positive isolates from Erasmus MC.