Comparative genomics for the identification of virulence factors in *Burkholderia cepacia*

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Introduction

Strains of *Burkholderia cepacia* are significant human pathogens, particularly causing infectious disease in patients with cystic fibrosis (CF). To date, the pathogenesis of *B. cepacia* infection is not well understood and few virulence factors have been identified. Nine genomovars of *B. cepacia* have been identified, each with distinct patterns of virulence and transmissibility. Genomovar III strains are the most predominant clinical species, being highly transmissible, highly virulent and associated with a poor prognosis in CF patients. Strains of genomovar II, also known as *Burkholderia multivorans*, are the second most prevalent in CF, whereas those of genomovar VII (*B. ambifaria*) are rarely encountered in clinical situations and are thought to be significantly less virulent. To complement the data being gathered through sequencing the genome of a genomovar III strain, and to identify regions associated with pathogenesis, a comparative genomic technique is proposed, in order to investigate differences between the genomes of *B. cepacia* from genomovars II, III and VII. Differential genomic DNA hybridisation[1] will be performed to isolate DNA which is uniquely present or absent in each strain. The prevalence of these regions within further clinical isolates will be investigated, and the regions characterised using either the genome sequence data or direct sequence analysis. The results of this may lead to the further investigation of putative virulence factors by site directed mutagenesis and infection model testing. In this way the information provided by the genome sequencing project can be employed and novel *B. cepacia* virulence genes can be rapidly investigated.

Methodology

To discover novel or variable genes in novel strain Y compared to sequenced strain X.

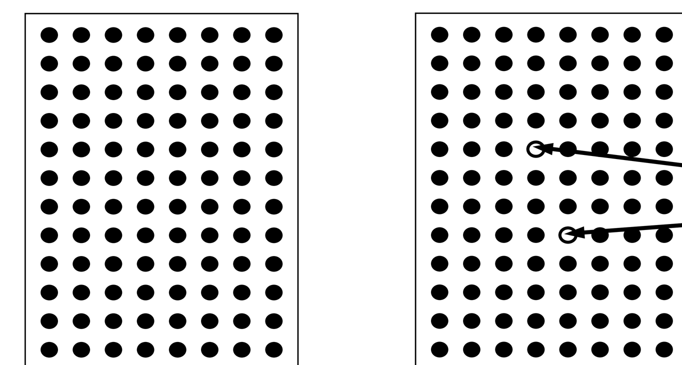
Create a 1kb library of strain X in pUC, with five times genome coverage.

Grid out the library onto nylon membrane at high density.

Picture courtesy of Richard Summers, Wellcome Trust Sanger Institute

High-throughput robotic machines are used in gridding.
This method should prove more comprehensive than subtractive PCR methods

Hybridise duplicate membranes in parallel with total genomic DNA of strain X, and with total genomic DNA of strain Y.



Negative clones identified through comparing membranes, indicating absence of specific fragment in strain Y. Clones sequenced at high-throughput and assembled

Interesting sequences will be analysed and used to probe BAC libraries for subsequent sequencing. Thus we can obtain the full novel sequence and determine the extent of potential islands.

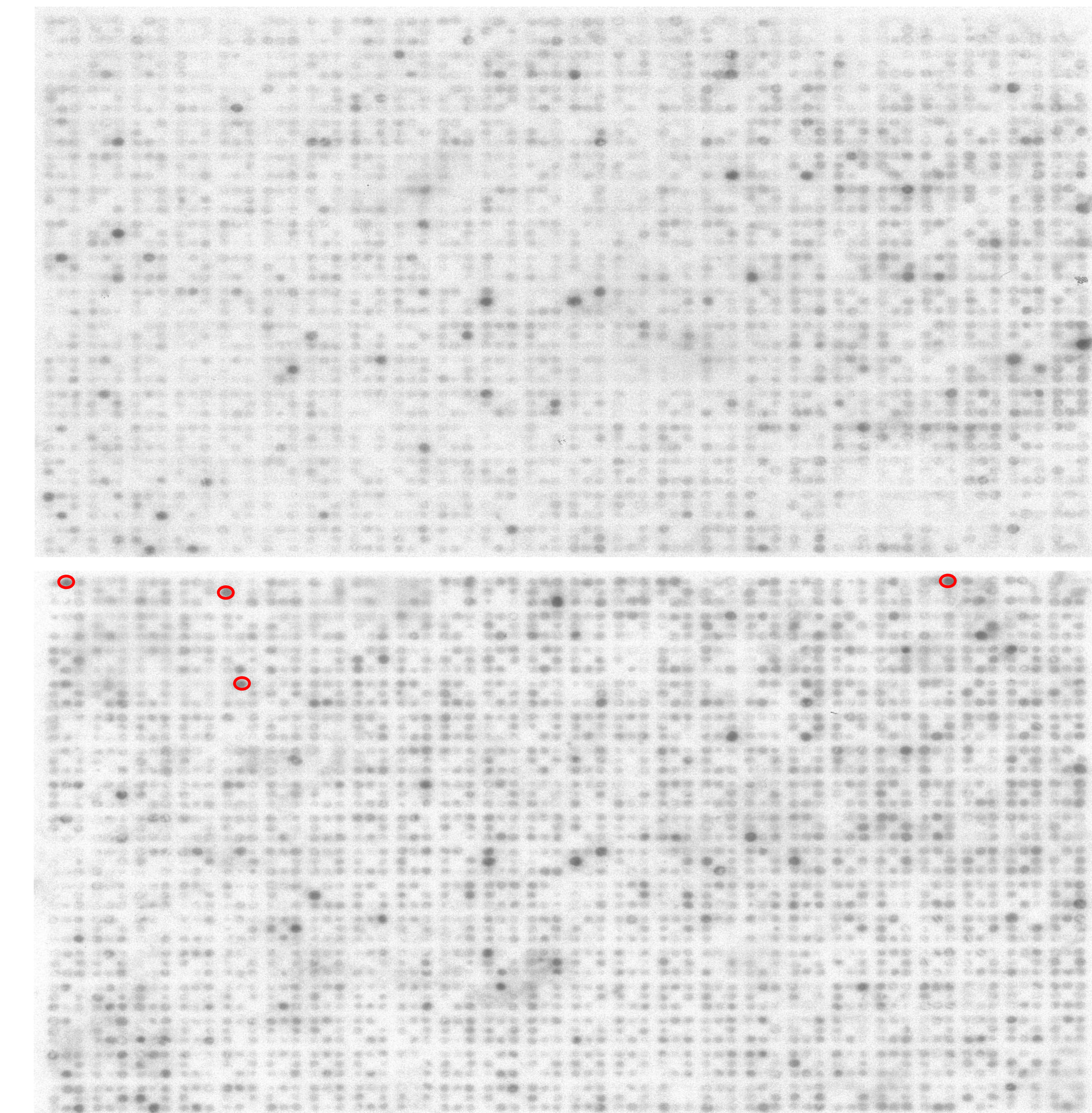
Depending on the hybridisation stringency, this technique will pick up entirely novel genes, or genes that are present in both strains, but with variable sequences.

Genes identified using this strategy can be used to probe genomic DNA of further strains to determine how wide-spread the gene/island is.

Strains under investigation:
Burkholderia cepacia (genomovar III) strain J2315[2] (sequenced strain: http://www.sanger.ac.uk/Projects/B_cepacia/)
Burkholderia multivorans (genomovar II) strain C1576[3]
Burkholderia ambifaria (genomovar VII) strain LMG 19182[4]

Example of results

Results obtained in ongoing project comparing strains of *Campylobacter jejuni*



Some of the clones with differential hybridisation patterns are indicated by red circles

Pictures courtesy of Emily Kay, Wellcome Trust Sanger Institute

References

1 **Liang, X., Pham, X.-Q.T., Olson, M.V. and Lory, S.** (2001) Identification of a genomic island present in the majority of pathogenic isolates of *Pseudomonas aeruginosa*. *J. Bacteriol.* **183**:843-853
2 **Govan, J.R.W., Brown, P.H., Maddison, J., Doherty, C.J., Nelson, J.W., Dodd, M., Greening, A.P. and Webb, A.K.** (1993) Evidence for transmission of *Pseudomonas cepacia* by social contact in cystic fibrosis. *The Lancet* **342:**15-19
3 **Whiteford, M.L., Wilkinson, J.D., McColl, J.H., Conlon, F.M., Michie, J.R., Evans, T.J. and Paton, J.Y.** (1995) Outcome of *Burkholderia* (*Pseudomonas*) *cepacia* colonisation in children with cystic fibrosis following a hospital outbreak. *Thorax* **50:**1194-1198
4 **Coenye, T., Mahenthiralingham, E., Henry, D., LiPuma, J.J., Laevens, S., Gillis, M., Speert, D.P. and Vandamme, P.** (2001) *Burkholderia ambifaria* sp. nov., a novel member of the *Burkholderia cepacia* complex including biocontrol and cystic fibrosis-related isolates. *Int. J. Syst. Evol. Microbiol.* **51:**1481-1490

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