Using insertion sequences to determine gene function in epidemic Pseudomonas aeruginosa

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Pseudomonas aeruginosa is the pathogen most commonly associated with morbidity and mortality in cystic fibrosis (CF). While most CF patients are infected with individual P. aeruginosa strains believed to be environmental in origin, in recent years strains that are transmissible from person to person have been emerging within CF clinics. These epidemic strains are often atypical, many are multi-drug resistant and some possess unusual characteristics such as an increased ability to aerosolise. The microbial factors responsible for these characteristics are not clear. We are using insertion sequences (IS) to investigate these factors, and to study the evolution of these strains. IS are mobile DNA elements possessing only the genes that allow them to move from one genomic site to another. By mapping IS to the genome we can determine genes necessary for movement and spread of P. aeruginosa. Intragenic insertion suggests that these particular genes are not crucial for cell survival since their transcription will be interrupted, whereas essential genes should not contain IS. Certain “active” IS can also act as promoters for downstream gene activation. A genome-based study was conducted to compare IS sites in the Liverpool and Manchester epidemic strains (LES and MAN) with those of three non-epidemic strains. All five strains had multiple IS. However, unlike the genomes of the non-epidemic strains, which had IS spread throughout the genome, MAN and LES had a propensity for IS clustering. The genes immediately downstream of two active IS in LES and MAN were determined and included homologs of glycosyl transferases and of genes involved in oxidoreductase activity. Supported by: The CF Trust, UK.

High bacterial diversity of Staphylococcus aureus populations in airway specimens of cystic fibrosis patients

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We analyzed the population diversity of Staphylococcus aureus in airway specimens of cystic fibrosis patients and in nasal colonizing situations. The heterogeneity of S. aureus was determined by quantifying the occurrence of phenotypic variants (differences in hemolysis, pigmentation, colony morphology) in primary cultures from nose, oropharyngeal and sputum specimens from CF patients and in nose swabs from healthy S. aureus carriers in 2 German Centers. The proportion of heterogeneous samples, the number of clearly distinguishable isolates per sample and the qualitative differences between phenotypes was significantly higher in CF spuata than in the other samples. The heterogeneity of the S. aureus population could be correlated with high bacterial densities in the sputum samples. Molecular analysis of heterogeneous samples by pulsed-field gel electrophoresis or spa-typing revealed that the bacteria were polyclonal (30%), monoclinal with minor genetic alterations (25%), or not distinguishable by the genetic typing methods used (69%). Importantly, differences in antibiotic susceptibility were detected in phenotypic S. aureus variants within a single specimen. Diversification of the S. aureus population is highly favored during chronic CF lung infection, supporting the general hypothesis that maintenance of intra-host diversity can be of adaptive value, increasing the fitness of the bacterial community. This work was partially funded by the German CF Foundation to BCK (Mukoviszidose e.V. Project F04/03) and CW (Mukoviszidose e.V. Project SH-42804–01).

Different genetic adaptation strategies of mucoid and non-mucoid Pseudomonas aeruginosa in the airway of cystic fibrosis patients

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Phenotypic and genotypic changes of infecting strains of P. aeruginosa play a critical role in the establishment and maintenance of chronic lung infections in patients with cystic fibrosis (CF). In the present investigation, GeneChip P. aeruginosa Affymetrix Genome Arrays were applied to determine the global transcriptional profiles of paired mucoid and non-mucoid P. aeruginosa isolates from early and late phases of chronic infection in four CF patients harbouring the same genotype. By comparing the gene expression patterns in isolates from different stages of the chronic lung infection, we found that the mucoid phenotype maintained an essentially unaltered transcriptional profile for decades, whereas substantial alterations of the transcriptional profiles were observed in corresponding isolates of the non-mucoid phenotype. The late non-mucoid isolates displayed reduced expression of many genes involved in quorum sensing, production of virulence factors and iron acquisition, and increased expression of genes involved in anaerobic respiration and antibiotic resistance as compared to the early isolates. In conclusion, the conserved gene expression pattern in the mucoid isolates versus the diversity of changes in non-mucoid P. aeruginosa isolates reflects different adaptation strategies used by these two phenotypes, possibly associated with the different requirements for survival in specific niches of the CF airways.

Comparison of T-RFLP profiles generated directly from sputum with those generated from conventional culture plates

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Routine microbiological analysis of CF sputum samples only provides data on the presence or absence of a small group of bacterial species. Recent molecular-based studies have suggested that the bacterial community in CF sputum is far more complex than previously believed, with a number of species candidates for inclusion in routine surveillance. Here we compare the T-RFLP profiles generated directly from 8 adult CF sputum samples with those generated from an array of standard culture plates from the same samples. The profiles generated from culture and sputum differed significantly, with a higher number of bands, and a greater phylogenetic range, resolved from the sputa. On average 14.4 (+10.8) bands were detected per sputum profile, compared with 8.5 (+5.4) bands per culture profile, with a total of 74 separate T-RF band lengths detected in the profiles arising directly from sputum, compared with 31 in the profiles arising from cultures. There were 78 instances of bands being detected in the T-RFLP profile from the sputum that were not detected in the profiles based on routine culture. In total, 60.5% of all the T-RF lengths detected were detected in sputum profiles alone. Further, there were significant differences in the predominance of species between the two sample types, with a total of 15 instances of T-RF bands detected in the sputum DNA profile alone that represented more than 10% of the total band volume. These data highlight the highly selective nature of culture-based microbial surveillance and suggest a thorough molecular-based survey of the bacterial species present in CF lung infections would be of great value in identifying previously unrecognised CF pathogens. Supported by: SPARKS.