104 Molecular analysis of diversity within the genus *Pseudomonas* in the lungs of cystic fibrosis patients

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Pseudomonas spp., especially *P. aeruginosa*, are important human pathogens and have been considered to be significant in the lung infections that Cystic Fibrosis (CF) patients suffer. Current methodologies that rely on in vitro cultivation of bacteria prior to analysis are either too cumbersome or lack sufficient discriminatory power to be able to define the diversity of *Pseudomonas spp*. in CF lung. This study was undertaken to differentiate species of the genus *Pseudomonas* by using a culture-independent molecular biological approach. This assesses the sequence diversity of the 16S-23S rDNA Internal Transcribed Spacer region 1 (ITS1) of the *Pseudomonas* species present in DNA extracted directly from CF sputum samples. In addition to the relevant controls, amplification of the ITS1 region was performed using 40 sputum samples. *Pseudomonas spp.* were detected in 75% of samples tested, including five samples from which no *Pseudomonas spp*. had been detected by conventional culture-based microbiological techniques. Following sequencing of individual clones, *in silico* analysis showed that most had the highest homology to the ITS1 region of *P. aeruginosa*. To allow a more rapid assessment, a Terminal Restriction Fragment Length Polymorphism (T-RFLP) based assay was devised for these PCR products. All the T-RFLP profiles obtained for this study set were indistinguishable from that generated for *P. aeruginosa*. The significance of these findings in relation to the wider bacterial community and clinical status of these patients is discussed.

105 Genomic fingerprinting of *Pseudomonas aeruginosa* isolates from all Swedish CF Centres. A Scandinavian Cystic Fibrosis Study Consortium study

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Objectives: Cystic fibrosis (CF) patients are often colonized with *Pseudomonas aeruginosa* (P.a.) and the majority by a strain acquired from the environment. Reports about cross-infection at CF Centres and camps have led to recommendations about segregation and regular microbiological surveillance. We aimed to conduct the first surveillance by genomic fingerprinting of P.a. isolates from all four Swedish CF Centres. More than 350 P.a. isolates from 208 CF patients were analyzed.
Method: P.a. isolates were genotyped using PFGE and the results were confirmed by MLVA and compared with a French P.a. collection.
Conclusions: Mainly unique strains for each patient were found. Clusters with strains shared by more than five patients were only found for B-, J- and X strains, which has been found in 22%, 14% and 5%, respectively. The B-strain is probably a common P.a. in the environment and has been isolated from patients in all four centres. Some strains have been isolated in both Sweden and France. The J-strain is shared by a few patients in three of four centres with indication of cross-infection at a camp. The B and X strains are assumed to be common in the environment, while the J-strain is multiresistant and probably more transmissible. The Swedish segregation routines seemed to have prevented frequent clustering and patient to patient transmission until 2004, except for patients attending a camp.
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106 Development of a diagnostic multiplex PCR test for the identification of three CF epidemic strains of *Pseudomonas aeruginosa*

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A number of CF epidemic strains of *Pseudomonas aeruginosa* have been reported in Europe and Australia, including the Liverpool epidemic strain (LES) and the Manchester epidemic strain (MES). A survey of CF centres in England and Wales showed that the LES was the most common clone amongst CF isolates (11% of all isolates), followed by the Midlands1 (Mid1) epidemic strain. Small clusters of the MES were also observed. Individual PCR amplification tests have been developed previously for the LES, Mid1 and MES.
We have developed a simple diagnostic multiplex PCR test that can be used routinely in laboratories on boiled suspensions of bacteria isolated from sputum. The test includes markers for the LES, Mid1 and MES strains and a 16S rRNA gene bacterial control.
In order to evaluate the test, we have screened collections of LES, MES and Mid1 isolates identified previously using pulsed field gel electrophoresis, along with various CF and non-CF non-epidemic *P. aeruginosa* strains. Since it is important to keep CF patients free from *P. aeruginosa* infection for as long as possible, it is necessary to monitor CF patients for the presence of possible transmissible strains. A simple PCR test should prove to be a useful tool for better informing clinicians charged with the responsibility for making decisions about segregation in order to prevent epidemic spread of *P. aeruginosa* strains amongst patients.
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107 Comparison of 6 culture media, 5 DNA-extraction methods and PCR and nested PCR for sensitive detection of *P. aeruginosa* from CF sputa

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We compared blood agar, McConkey agar, cetrimide agar, cetrimide broth, *Pseudomonas* selective agar, *Pseudomonas* minimal medium for the detection of *P. aeruginosa* from a serial fivefold dilution of *P. aeruginosa* positive sputa into *P. aeruginosa* negative sputa.
On the same serial dilution series we compared the QiaGen Tissue kit, Roche High Pure PCR Template Preparation kit, Roche MTB DNA extraction kit, Whatman DNA Extraction Paper and Looxster DNA Extraction kit for use of the purified DNA in a *P. aeruginosa* OprD gene specific PCR and nested PCR.
The most sensitive culture approach was found to be cetrimide broth incubation during 24 hours and subsequent plating onto blood agar. The Roche High Pure PCR kit was found superior to the other techniques for DNA extraction and in combination with nested PCR a further two steps of the serial dilution (i.e. a 25 fold dilution) became positive compared to cetrimide broth culture based detection.