Rapid identification of Stenotrophomonas maltophilia by peptide nucleic acid fluorescence in situ hybridization

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Abstract

The objective of this study was to design and evaluate a novel peptide nucleic acid (PNA) probe for S. maltophilia identification by fluorescence in situ hybridization (FISH). A PNA probe targeting 16S rRNA (Flu-OO-CGCCATG-GATGTTC, 5'-3') specific for S. maltophilia and conjugated with fluorescein isothiocyanate (FITC) was added a PNA blocker probe (Ac-CCACGGATGTTCC, 5'-3') to prevent cross-reaction with closely related species such as Xanthomonas campestris (Panagene, Daejeon, Korea). Sensitivity and specificity of the probe were evaluated using 35 human and veterinary clinical S. maltophilia isolates, and 43 reference and clinical strains representing common bacterial species in the airways of humans and animals (Table I). All strains were cultured on solid media (5% calf blood agar, chocolate agar or nutrient/yeast/glycerol agar as deemed appropriate) and cultured on solid media (5% calf blood agar, chocolate agar or nutrient/yeast/glycerol agar as deemed appropriate) and sub-cultured in tryptic soy broth. Ten microliters of broth were placed onto a microscopic slide prepared with one drop of fixation solution (phosphate-buffered saline with detergent) and fixed by heating (methanol fixation for sputum smears). After adding one drop of hybridization solution (AdvanDx, Woburn, MA, USA) containing the S. maltophilia PNA probe, a coverslip was applied and hybridization performed by incubating the slides at 55°C for 30 min (90 min for sputum smears). Limited to Gram-negative strains, slides were immersed in preheated deionized water (55°C) for 1 min. All slides were then placed in a wash jar with a preheated (55°C) wash solution (diluted Tris-buffered saline with detergent) in a water bath for 30 min, coverslips removed. After air drying, a drop of mounting medium (photobleaching inhibitor in glycerol) and a coverslip were applied. A positive control slide with S. maltophilia and a negative control slide with the relevant strain hybridized with a universal PNA FISH probe (BacUni PNA, AdvanDx) according to manufacturer’s instructions were included in all runs. All slides were evaluated within 2 h under a fluorescence microscope (×60 objective, Olympus BX51, Ballerup, Denmark; Mercury U-LH100HG 100 W lamp) equipped with an FITC/Texas Red Dual Band Filter. Fluorescence images were obtained using an Olympus DP72 camera (1360 × 1024 pixels, 1 s exposure). Samples were considered positive when single cells had a strong fluorescence and clear...
maltophilia microscopy of the smears showed at least one to ten S. maltophilia cells in most fields when the concentration in the sample was $10^5$ CFU/mL. The probe specificity was further tested on sputum samples from seven patients with CF. The samples underwent routine culture at the University Hospital of Copenhagen, Denmark. The probe produced positive results in all four samples from S. maltophilia-infected patients and negative results in all control samples (two patients infected with Pseudomonas aeruginosa, one patient infected with Burkholderia multivorans). No background fluorescence was observed.

Isolation and identification of S. maltophilia can be problematic [9–13]. Selective differential media have been recommended for improved detection of S. maltophilia from non-sterile sites such as respiratory secretions [14–16]. Problems related to misidentification of S. maltophilia by phenotypic methods can be overcome by the use of molecular methods. Pinot et al. [17] used vancomycin, imipenem, amphotericin B medium agar for isolation and multiplex PCR for identification of S. maltophilia. Hogardt et al. [18] designed a species-specific DNA probe for S. maltophilia identification and demonstrated that the probe could be used successfully on sputum and throat samples from CF patients. However, the limit for microscopic detection of bacteria within sputum was $4 \times 10^5$ CFU/mL and the sensitivity of the DNA FISH method (90%) was lower than that of the PNA FISH approach described in this study. PNA probes are small in size with a hands-on time. Furthermore, FISH is useful for in situ detection of this microorganism directly in clinical samples and mixed bacterial populations without prior cultivation. Thus, the S. maltophilia PNA FISH probe described in this study has
important applications for studies of biofilm infections and *S. maltophilia* colonization in patients with CF, where colonization and chronic infection with *S. maltophilia* is commonly reported.

In conclusion, the *S. maltophilia* PNA FISH probe demonstrated excellent sensitivity and specificity when tested against clinically relevant bacteria occurring in the respiratory tract of humans and animals. The PNA FISH assay can be implemented in diagnostic laboratories for rapid, simple and reliable identification of *S. maltophilia* in clinical specimens. It can only be a valuable tool for research aimed at understanding the role played by this organism in CF and in equine respiratory tract infections. For this purpose, further studies are warranted to evaluate the use of the probe for studying spatial distribution in polymicrobial biofilms.

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**Conflict of Interest**

None declared.

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