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Rapid identification of *Fusobacterium nucleatum* and *Fusobacterium necrophorum* by fluorescence in situ hybridization

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Abstract

Identification of clinically relevant *Fusobacterium* spp. is hampered by their slow growth, their frequent occurrence in polymicrobial culture, and the low reliability of biochemical differentiation methods. A newly developed fluorescence in situ hybridization (FISH) assay allowed reliable and rapid identification of *Fusobacterium necrophorum* and *Fusobacterium nucleatum* from culture. Preliminary results show that the method offers the perspective for direct detection of these pathogens in blood cultures and abscess aspirates.

Keywords: *Fusobacterium*; *Fusobacterium nucleatum*; *Fusobacterium necrophorum*; FISH; 16S rRNA; Probe

Fusobacteria are obligate anaerobic, slender, or large pleomorphic Gram-negative rods. The genus *Fusobacterium* currently comprises 13 species, but the taxonomy is still in a state of change (Citron, 2002; Conrads et al., 2002). Fusobacteria are part of the physiologic flora, especially of the oropharyngeal and gastrointestinal tract, but *Fusobacterium nucleatum* and *Fusobacterium necrophorum* in particular may cause manifold infections such as periodontitis, abscesses in various organs, bacteremia, and postanginal sepsis syndrome (Jousimies-Somer et al., 1993; Smith and Thornton, 1993; Batty and Wren, 2005; Nadkarni et al., 2005; Blairon et al., 2006). The remaining species are rare causes of intra-abdominal infections (e.g., *Fusobacterium varium*), animal bite infections (e.g., *Fusobacterium russii*), or tropical ulcer (e.g., *Fusobacterium ulcerans*) (Smith and Thornton, 1993). The identification of *Fusobacterium* spp. is complicated by their slow growth rate and frequently occurring concomitant flora. Typical colony and microscopic morphology as well as biochemical differentiation by the API systems (bioMerieux, Nürtingen, Germany) are often used for presumptive identification of *Fusobacterium* spp. (Tanner et al., 1985; Baron and Citron, 1997), but biochemical identification is not always reliable according to previous reports (Summanen and Jousimies-Somer, 1988; Downes et al., 1999). Definitive species identification, which is essential when isolates from blood, spinal fluid, or deep organs are investigated, requires time-consuming and costly methods such as fermentation tests, cell wall fatty acid profiling, or molecular methods such as 16S rRNA sequencing (Moore et al., 1994; Tanner et al., 1994). Because of these difficulties, PCR-based methods and other molecular methods such as DNA–DNA hybridization (Jervoe-Storm et al., 2005; Store et al., 2005) have been developed. Fluorescence in situ hybridization (FISH) has been implemented for some special purposes, that is, to visualize *F. nucleatum* in biofilms in vitro, to detect fusobacteria in patients with gingivitis, or to quantify *Fusobacterium prausnitzii* in stool (Suau et al., 2001; Sunde et al., 2003; Gmur et al., 2004, Foster and Kohlenbrander, 2004). FISH using fluorescently labeled rRNA-targeted oligonucleotide probes has become a useful diagnostic tool for rapid identification of pathogens in blood cultures and cerebrospinal fluid (Jansen et al., 2000; Poppert et al., 2005; Wellinghausen et al., 2006). It also allows the diagnosis of bacteria that are slow growing or difficult to cultivate (Trebesius et al., 2000; Poppert et al.,

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2002). However, the usefulness of FISH for the identification of *Fusobacterium* spp. has never been thoroughly evaluated on sufficiently large strain collections. In our hands, 2 of the published probes JF3 5′-CCC TAA CTG TGA GGC AAG (Foster and Kohlenbrander, 2004) and FUSO 5′-CTA ATG GGA CGC AAA GCT CTC (Sunde et al., 2003) did not stain 22 of 22 and 7 of 45, respectively, of the target strains (data not shown).

Therefore, we designed a new hierarchical set of 3 16S rRNA-targeted oligonucleotide probes for the identification of *Fusobacterium* spp., particularly the clinically most relevant species *F. nucleatum* and *F. necrophorum*. The sensitivity and specificity of the FISH assay were first evaluated on 85 characterized target strains and nontarget strains, including 47 clinical isolates of *Fusobacterium* spp. In the next step, the evaluated FISH assay was directly implemented in 4 positive anaerobic blood cultures of 4 neutropenic patients with hematologic malignancies and in 4 peritonsillar abscess aspirates showing slender or pleomorphic Gram-negative rods with Gram stain. Three of the 4 abscesses were polymicrobial. FISH probes were designed using the ARB software package, available at http://www.arb-home.de. The first probe targets all *Fusobacterium* spp. (Fus all 307, 5′-TCA GTC CCC TTG TGG CCG) and *Leptotrichia* spp., a close relative that is infrequently isolated from blood cultures of patients with hematologic malignancies (Blairon et al., 2006). Because of the intention to target all *Fusobacterium* spp. and *Leptotrichia* spp., it was not possible to avoid the probe cross-reaction with *Streptobacillus moniliformis*, a rarely isolated pathogen that causes rat-bite fever (Andre et al., 2005). The *F. nucleatum* cluster probe (Fus nuc 611, 5′-CGC AAT ACA GAG TTG AGC CCT GC) targets *F. nucleatum*, including its 5 subspecies, *F. nucleatum* subsp. *animalis*, *fusiforme*, *nucleatum*, *polymorphum*, and *vincentii*, and the 4 genetically closely related species, *Fusobacterium simiae*, *Fusobacterium naviforme*, *Fusobacterium periodenticum*, and *Fusobacterium canifelinum* (formerly *F. nucleatum* subsp. *canifelinum*), which form one cluster according to internal transcribed spacer sequencing (Conrads et al., 2002). The probe cross-reaction with the Gram-positive anaerobe *Filifactor alocis* (formerly *Fusobacterium alocis*) was unavoidable because of close genetical relationship, but all mentioned species except *F. nucleatum* are rarely associated with human infections (Citron, 2002, Conrads et al., 2002, Kumar et al., 2006). The third probe is species specific for *F. necrophorum* (Fus nec 999, 5′-CGC ATC TCT GCT CCG TTC GTA). Probes were synthesized and directly 5′-labeled with FITC or Cy3 (Thermo Hybaid, Germany). The eubacterial probe EUB338 (Amann et al., 1990) was used

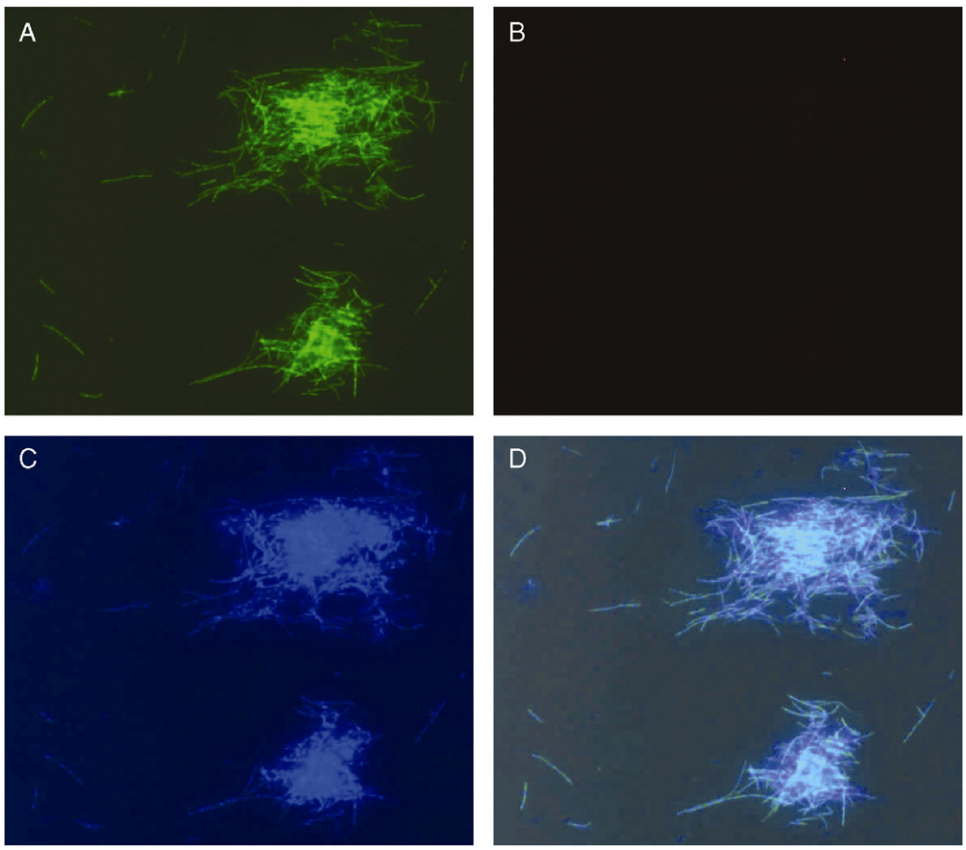

Fig. 1. Identification of *F. nucleatum* from culture by FISH. The *F. nucleatum* strain was stained by the FITC-labeled eubacterial probe (EUB 338) (A) in combination with the Cy3-labeled probe Fus nec (B), which was negative, and DNA stain DAPI (C). The overlay (D) demonstrates that the bacteria fluoresce in the FITC and DAPI channels.