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Development of real-time polymerase chain reaction assay for specific detection of *Tsukamurella* by targeting the 16S rRNA gene

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Abstract

Recently, members of the genus *Tsukamurella* have been implicated as important etiologic pathogens contributing to bloodstream and pulmonary infections in immunocompromised patients. *Tsukamurella* species share many features with other mycolic acid–containing genera of the order *Actinomycetales* and might therefore be misidentified as belonging to one of these genera. We developed a TaqMan-based real-time polymerase chain reaction assay for the rapid and specific detection of infections due to *Tsukamurella* species. The assay amplifies and detects a 157-bp segment of the 16S rRNA gene of *Tsukamurella*. The specificity of the assay was confirmed using a panel of DNAs from 12 *Tsukamurella* strains and 11 strains belonging to 8 phylogenetic closely related genera. The sensitive and specific nature of the assay provides a valuable tool for the early and precise diagnosis of *Tsukamurella* infections in clinical diagnostic laboratories.

Keywords: Real-time PCR; *Tsukamurella*; Mycolic acid–containing taxa

1. Introduction

The name *Tsukamurella* was introduced by Collins et al. (1988) for organisms classified as *Corynebacterium paurometabolum* and *Rhodococcus aurantiacus*. *Tsukamurella* species are obligate aerobic, Gram-positive, partially acid-fast, nonmotile bacilli. The genus *Tsukamurella* belongs to the order *Actinomycetales* and, phylogenetically, is closely related to the genera *Corynebacterium*, *Dietzia*, *Gordonia*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Segniliparus*, and *Williamsia*. *Tsukamurella* species are environmental saprophytes found in soil, sludge, and could be isolated from arthropods (McNeil and Brown, 1994). They are known to be opportunistic pathogen causing serious infections in humans, specifically in patients with predisposing conditions, such as immunosuppression due to lymphoid and solid neoplasms, organ transplantation, HIV infection, chronic pulmonary diseases, use of corticosteroid, and in patients who are carrying foreign bodies such as long-term indwelling central venous catheters. The genus *Tsukamurella* currently contains 12 species with validly published names. Species recovered from clinical specimens include *Tsukamurella inchonensis* (Yassin et al., 1995), *T. pulmonis* (Yassin et al., 1996), *T. paurometabola* (Collins et al., 1988; Steinhaus, 1941), *T. strandjordii* (Kattar et al., 2001), and *T. tyrosinosolvens* (Yassin et al., 1997). Although early reported cases of serious illness in humans were identified to be due to *T. paurometabola* (Auerbach et al., 1992; Casella et al., 1987; Granel et al., 1996; Jones et al., 1994; Lai, 1993; Shapiro et al., 1992), our recent review of the literature (from 1998 to 2011 examining 20 reported cases), and after the recognition of other *Tsukamurella* species, indicates *Tsukamurella tyrosinosolvens* to be the most frequent cause of *Tsukamurella* infections followed by *Tsukamurella pulmonis*. The last 2 species have predilection for infecting critically ill patients, with bloodstream infections (BSI) and pneumonia being the most common infection types.

Tsukamurella species share many phenotypic characteristics with other species of the mycolic acid–containing genera of the order *Actinomycetales* and, therefore, might be misidentified as belonging to one of these genera when standard biochemical tests, e.g., the API Coryne System

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(API-bioMérieux, Inc., La Balme les Grottes, France), were used for their identification (Almuzara et al., 2006). The similarity of *Tsukamurella* to other more common pathogens expected in immunocompromised patients, such as *Mycobacterium*, may have previously resulted in the underreporting of this genus. Therefore, a diagnostic strategy that is both sensitive for detection of *Tsukamurella* disease and specific for its exclusion across all forms of the disease is required. This is a critically important component of antibiotic therapy because it can reduce the cost of treatment and toxicity and prevent the emergence of drug-resistant strains.

The advent of molecular assays for diagnosis of pathogenic microorganisms opened up a new era in the microbiological laboratory. The technology allows rapid and accurate identification of the etiologic agent in a time shorter than that of traditional methods. This allows for earlier initiation of a focused chemotherapy and decreases the likelihood of disease progression. Molecular diagnostic methods using polymerase chain reaction (PCR) are considered now as the gold standard in clinical diagnostics of bacterial infections. Most rely on the amplification of parts of the 16S rRNA gene sequence. To facilitate the identification of *Tsukamurella* infections, we report a probe-based real-time PCR assay to expedite the detection of *Tsukamurella*. This assay provides a novel, straightforward strategy for the detection of this important group of emerging pathogens.

2. Materials and methods

2.1. Bacterial strains

To evaluate whether the developed real-time PCR is specific for *Tsukamurella*, 23 bacterial strains were used (Table 1). Twelve strains representing 5 clinically relevant *Tsukamurella* species and the other 11 strains were chosen because they represent phylogenetically related genera with pathogenic potential. The type strains of *Tsukamurella* species as well as the clinical isolates were isolated by us and stored in our culture collection (IMMIB). The reference strains of *Tsukamurella paurometabola* DSM 20162^T, *Rhodococcus globerulus* DSM 43956^T, and *Dietzia maris* DSM 43672^T were obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig Germany); the type strain of *Tsukamurella strandjordii* ATCC BAA-173^T was generously provided by M.B. Coyle; and *Mycobacterium microti* NCTC 8710^T was obtained from the National Collection of Type Cultures, Central Public Health Laboratory, London. All strains were grown on Columbia blood agar (Becton Dickinson, Heidelberg, Germany) and were identified by standard biochemical tests as described previously (Yassin et al., 1995) and by using 16S rRNA gene sequence analysis. *Mycobacterium gordonae* and representatives of the *Mycobacterium tuberculosis* complex examined in this study (*M. tuberculosis*, *M. bovis*, *M. microti* NCTC 8710^T) were grown on Löwenstein-Jensen medium and, besides phenotypic (biochemical and thin-layer chromatography) and 16S rRNA gene sequencing identification, were examined using the Genotype Mycobacterium CM/AS Molecular Genetic Assay (Hain Lifescience, Nehren, Germany), in accordance with the manufacturer's instructions.

Table 1
Species used to test the analytical specificity of the real-time PCR for *Tsukamurella* species

Bacterial species	Culture collection number	Source
Tsukamurella inchonensis	ATCC 700082^T	Blood culture
T. inchonensis	IMMIB Al-1155	Smear from lung
T paurometabola	DSM 20162^T	DSMZ
T. pulmonis	ATCC 700081^T	Sputum
T. pulmonis	Clinical isolate	Sputum
T. pulmonis	Clinical isolate	Sputum
T. pulmonis	Clinical isolate	Sputum
T. strandjordii	ATCC BAA-173^T	Blood culture
T. tyrosinosolvens	DSM 44234^T	Blood culture
T. tyrosinosolvens	Clinical isolate	Blood culture
T. tyrosinosolvens	Clinical isolate	Blood culture
T. tyrosinosolvens	Clinical isolate	Blood culture
Corynebacterium mucifaciens	Clinical isolate	Blood culture
Dietzia maris	DSM 43672^T	DSMZ
Gordonia terrae	Clinical isolate	Sputum
Mycobacterium bovis	Clinical isolate	Sputum
Mycobacterium gordonae	Clinical isolate	Sputum
Mycobacterium microti	NCTC 8710^T	Vole isolate
Mycobacterium tuberculosis	Clinical isolate	Sputum
Nocardia elegans	DSM 44890^T	Sputum
Rhodococcus globerulus	DSM 43956^T	DSMZ
Segniliparus rotundus	Clinical isolate	Sputum
Williamsia serinedens	Clinical isolate	Blood culture

2.2. Genomic DNA extraction and 16S rRNA gene sequence determination

For the isolation of genomic DNA, the strains were grown on Columbia blood agar plates supplemented with 5% sheep blood at 35 °C (*Williamsia serinedens* was grown at 28 °C) for 48 h, whereas the mycobacteria were grown on Loewenstein-Jensen at 37 °C until visible growth. A loopful of pure culture was suspended in 400 μL of saline-EDTA to lyse the cells, and genomic DNA was isolated and purified using the Prep-A-Gene purification kit (BioRad, Munich, Germany) as described by the manufacturer. PCR-mediated amplification of the 16S rRNA gene was carried using procedures described previously (Rainey et al., 1996). The purified PCR products were sequenced using a *Taq* DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems, Darmstadt, Germany) as described by the manufacturer.

2.3. Probe and primer design

Probe design was performed by computer-aided comparative analysis of ssu-rRNA sequence datasets using the PROBE_DESIGN tool included in the software package