

## NOTES

# Organisms Designated as *Nocardia asteroides* Drug Pattern Type VI Are Members of the Species *Nocardia cyriacigeorgica*<sup>∇</sup>

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***Nocardia cyriacigeorgica* has recently been described as an “emerging” pathogen. However, DNA-DNA hybridization results confirm that *Nocardia asteroides* drug pattern type VI, which has long been recognized as a common and significant pathogen in the United States, belongs to the species *N. cyriacigeorgica*.**

*Nocardia cyriacigeorgica* has recently been described as a “new” species of *Nocardia* capable of causing disease in humans (24). The type strain of the species was recovered from bronchial secretions of a patient with chronic bronchitis. 16S rRNA gene sequencing showed this isolate to be distinct from 17 validly described species of *Nocardia*; DNA-DNA hybridization showed it to be unrelated to *N. abscessus* (presumably DSM 44432<sup>T</sup>) (45% reassociation) and to the *Nocardia asteroides* type strain (presumably ATCC 19247<sup>T</sup>) (61% reassociation) (24).

In 1988, Wallace et al. described variations in the drug susceptibility patterns of 78 isolates determined biochemically to be *N. asteroides* (22). Most of these 78 isolates fell into one of six different susceptibility patterns; an additional miscellaneous drug pattern group included the type strain of *N. asteroides* (ATCC 19247<sup>T</sup>). Based largely on molecular testing, members of drug pattern types I, III, and V have subsequently been determined to belong to *N. abscessus*, *N. nova*, and *N. farcinica*, respectively. In the 1988 study of Wallace et al., isolates determined to belong to *N. asteroides* drug pattern type VI showed resistance to the penicillins and susceptibility to the broad-spectrum cephalosporins. *N. asteroides* drug pattern type VI has not been assigned a distinct species name; ATCC 14759 has been designated a reference strain for this drug pattern group (20).

Recently, several authors have noted the genetic similarity of *N. cyriacigeorgica* and *N. asteroides* drug pattern type VI, by using sequence analysis of the 16S rRNA gene (2, 6, 7, 16, 18) and the 65-kDa heat shock protein (HSP) gene (17), and have commented that these may in fact be the same species. To our knowledge, conclusive information concerning the conspecificity of these organisms, as determined by DNA-DNA hybridization, has not been reported.

We report here the results of sequence analysis and DNA-DNA hybridization tests of the reference strain of *N. asteroides* drug pattern type VI and the type strain of *N. cyriacigeorgica*.

**Organisms.** The reference strain of *N. asteroides* drug pattern type VI (ATCC 14759) and the type strain of *N. cyriacigeorgica* (DSM 44484<sup>T</sup>) were examined in this study.

**Molecular methods.** DNA was extracted for amplification and gene sequencing, as previously described (8). Sequencing of the 16S rRNA gene, the HSP gene, and the *secA1* gene was performed as previously described (7, 8, 10). The deduced amino acid sequences of the HSP and *secA1* genes for both isolates were determined with Megalign software (DNA Star, Inc., Madison, WI). Genomic DNA was extracted from both organisms, and DNA-DNA hybridization was performed as previously described (3, 4, 9). DNA-DNA hybridization was performed twice with *N. cyriacigeorgica* as the source of the labeled DNA and once with *N. asteroides* drug pattern type VI as the source of the labeled DNA. All reactions were performed in duplicate at 70°C. The relative binding ratio (RBR) [(percentage of heterologous DNA bound to hydroxyapatite/percentage of homologous DNA bound to hydroxyapatite) × 100] was calculated by the method of Brenner et al. (3). The percent divergence (calculated to the nearest 0.5%) was determined by assuming that each degree of heteroduplex instability, compared to the melting temperature of the homologous duplex, was caused by 1% unpaired bases (3).

Sequence analysis of 1,384-bp regions of the 16S rRNA gene showed the reference strain of *N. asteroides* drug pattern type VI to be identical to the type strain of *N. cyriacigeorgica*. The HSP gene sequence of *N. asteroides* drug pattern type VI shows 99.5% sequence similarity to that of *N. cyriacigeorgica*, with two base differences in a 373-bp region. The deduced amino acid sequences of the HSPs of the two organisms were identical. Comparison of a 465-bp region of the *secA1* gene showed the two organisms to have 99.1% sequence similarity (four base differences); a similarity of >99.0% with this gene has been shown to indicate species identity (10). The deduced amino acid sequence of the SecA1 protein of *N. asteroides* drug pattern type VI was identical to that of *N. cyriacigeorgica*. DNA-

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TABLE 1. DNA relatedness of the reference strain of *N. asteroides* drug pattern type VI (ATCC 14759) to *N. cyriacigeorgica* (DSM 44484<sup>T</sup>)

Source of unlabeled DNA	Source of labeled DNA	RBR (% divergence)
<i>N. asteroides</i> drug pattern type VI	<i>N. cyriacigeorgica</i>	89.5 (3.5)
<i>N. asteroides</i> drug pattern type VI	<i>N. cyriacigeorgica</i>	82.7 (2.0)
<i>N. cyriacigeorgica</i>	<i>N. asteroides</i> drug pattern type VI	76.0 (2.5)

DNA hybridization studies showed the organisms to be related, with an RBR of greater than or equal to 76.0 and percent divergence of less than or equal to 3.5 (Table 1). These values are well within the ranges established for species identity (19, 23). Thus, by currently accepted standards, *N. cyriacigeorgica* and isolates described as *N. asteroides* drug pattern type VI belong to the same species.

Species formerly referred to as the “*N. asteroides* complex” are a heterogeneous group of organisms that are generally nonreactive on hydrolysis media and show few differences in other biochemical characteristics (5, 18). Numerous authors studying the similarities of isolates of “*N. asteroides*” have noted variations in susceptibility to various antibiotics (12, 13, 22) and in cell wall fatty acid components (15). Sequence analyses of “*N. asteroides*” isolates have confirmed the heterogeneity of isolates formerly assigned to this species and now formally designated distinct species (18).

Isolates belonging to *N. asteroides* drug pattern type VI are similar to each other in susceptibility to amikacin, cefamandole, cefotaxime, ceftriaxone, and imipenem and resistance to amoxicillin-clavulanic acid, ampicillin, ciprofloxacin, clarithromycin, and erythromycin (5). This organism, now named *N. cyriacigeorgica*, appears to be a common pathogen worldwide. In the 1988 study by Wallace et al., isolates belonging to *N. asteroides* drug pattern type VI comprised 35% of the 78 “*N. asteroides*” isolates examined. Since then, organisms of this type have been described as the most commonly isolated drug pattern type of “*N. asteroides*” in the southern United States (5). *N. cyriacigeorgica* is similarly described as a frequently isolated species in Japan (10% of 303 *Nocardia* isolates) and in Thailand (23.5% of 96 *Nocardia* isolates), as determined by chemotaxonomic and phenotypic methods. Using sequence analysis, an additional study of 121 isolates identified as “*N. asteroides*” from Japan and Thailand showed 27 of these isolates (22.3%) to have 16S rRNA gene sequences identical to that of the type strain of *N. cyriacigeorgica*. In addition, isolates of *N. cyriacigeorgica* have been identified by 16S rRNA gene sequence analysis from patients with disseminated disease in Canada, France, Greece, The Netherlands, and Turkey (1, 2, 11, 14, 21).

*N. cyriacigeorgica*, therefore, is not a “new” organism or an “emerging” pathogen; rather, it is a newly named but long-recognized agent of human disease.

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