

Short Report

Mitochondrial DNA Analysis of *Sporothrix schenckii* from China, Korea and Spain

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Abstract

Sporothrix schenckii isolates from China, Korea and Spain were investigated for mtDNA types based on restriction fragment length polymorphism patterns with *Hae*III. Sixty-one isolates from China, 8 from Korea and 11 from Spain were comprised of 7, 2 and 2 mtDNA types, respectively. All the isolates belonged to Group B.

Key words: *Sporothrix schenckii*, molecular epidemiology, mitochondrial DNA, China, Korea, Spain.

Introduction

Sporothrix schenckii is a causative agent of sporotrichosis and is distributed worldwide. Restriction fragment length polymorphism (RFLP) in mitochondrial DNA (mtDNA) has been useful for identifying, typing and grouping *S. schenckii*¹⁻⁶, and for clarifying its epidemiology. Moreover, RFLP is essential for identifying environmental isolates of *S. schenckii*⁷. To date, the isolates have been classified into 24 mtDNA types (Types 1-24) based on RFLP patterns obtained with *Hae*III (Fig. 1). The types have been phylogenetically classified into Group A (Types 1-3, 11, 14-19, 22 and, 23) and Group B (Types 4-10, 12, 13, 20, 21 and, 24)⁶. Although Mora-Cabrera *et al.*⁸ recently reported 6 new mtDNA types for clinical isolates from Mexico, Guatemala and Columbia, as 3 of them have RFLP patterns that are very similar to mtDNA types already reported⁶ they are being re-examined at Kanazawa Medical University.

mtDNA analysis has shown that the types and groups are related to geographic origin. Group B is predominant in Australia, Japan and China, while Group A is predominant in South Africa, North America, Central America and South America²⁻⁶. For example, 67 clinical isolates in China⁵ were composed of 5 mtDNA types: 58 isolates of Type 4; 1 of Type 5; 5 of Type 6; 1 of Type 20 and 2 of Type 24 based on RFLP patterns obtained with *Hae*III. All the isolates belonged to Group B, and 87% of them were Type 4. However, all the isolates were obtained from just one district of China-in the northeast.

The above molecular epidemiological findings were supported by polymerase chain reaction (PCR) single-strand conformation polymorphism (SSCP) analysis of each mtDNA type done by Sugita⁹. In addition, Mesa-Arango *et al.*¹⁰ used random amplified polymorphic DNA (RAPD) analysis to show a relationship between genotypes and geographical origins of isolates, from Mexico, Guatemala and Colombia, but they did not carry out mtDNA analyses.

Herein, we report mtDNA types of more

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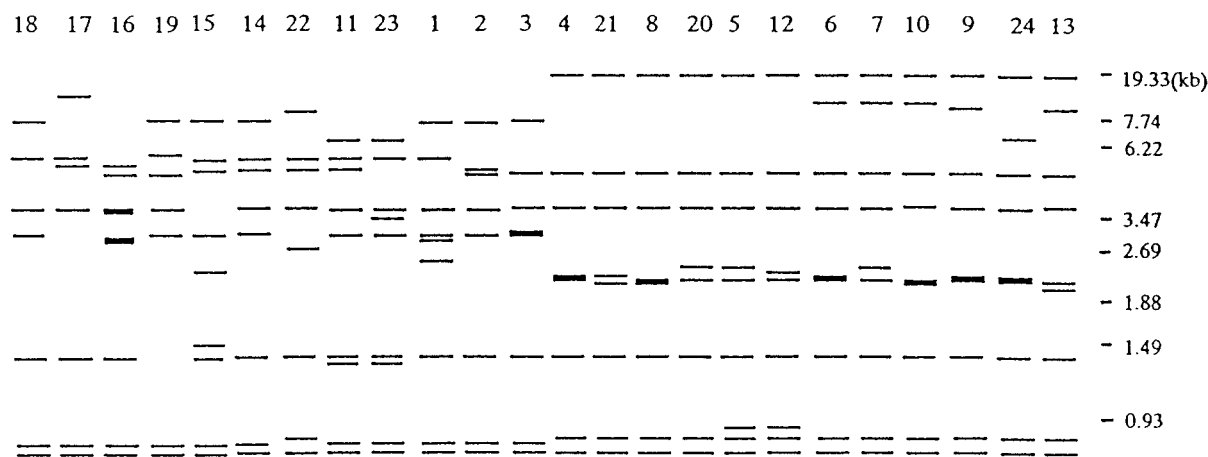


Fig. 1. A schematic representation of the mtDNA-RFLP patterns with *Hae*III.

The number at the top indicate the mtDNA types (cited from reference 5).

clinical isolates: 61 from China; 8 from Korea; and 11 from Spain.

mtDNA was prepared as described previously²⁾. In brief, *S. schenckii* cells were disrupted by sonification after treatment with Zymolyase-100T in sorbitol buffer. The mitochondrial fraction was obtained by centrifugation at 1,600 ×g for 10 minutes, followed by centrifugation of the supernatant at 20,000 ×g for 10 minutes. mtDNA was extracted from the mitochondria by phenol and then precipitated by addition of ethanol.

RFLP patterns were obtained using *Hae*III, *Hha* I, *Msp* I, *Bgl* II, and *Eco*RV, and analyses as described previously⁵⁾.

The 61 isolates from China were obtained from northeast, central, western and southern areas of China. They comprised 7 mtDNA types: 41 isolates of Type 4; 1 of Type 5; 4 of Type 6; 4 of Type 7; 2 of Type 9; 4 of Type 10; and 5 of Type 20 (Fig. 2). All the isolates belonged to Group B. Types 7, 9 and 10, were found for the first time in China, bringing the total to 8. About 70% of the isolates were Type 4, and so there is agreement with a previous report that Type 4 isolates are dominant⁵⁾. These findings indicate that the mtDNA types in China and Japan are distributed similarly.

The 8 isolates from Korea were 5 of Type 4 and 3 of Type 6, which are Group B. Not only do the isolates from Korea and China belong to Group B, those from Japan also do, and it is worth noting that Japan used to be attached to the continent.

The 11 isolates from Spain comprised 6 isolates of Type 5 and 5 isolates of Type 20. This was the first mtDNA analysis of European isolates, and although the number of isolates

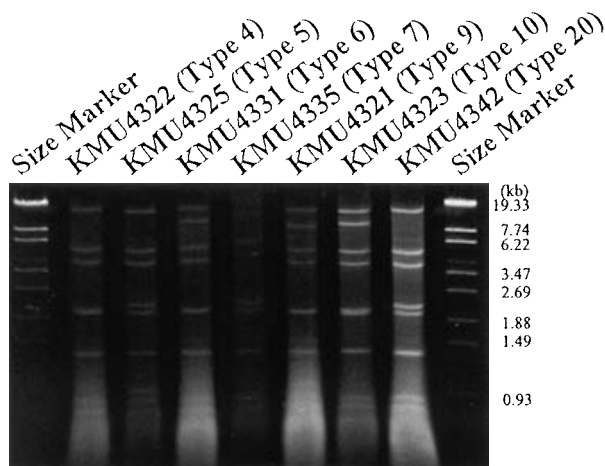


Fig. 2. Seven representative mtDNA-RFLP patterns of Chinese isolates with *Hae*III.

was small, it is interesting that they all belonged to Group B. Type 4, which is dominant in China, Korea, Japan and Australia; none, however, were found in Spain. Therefore, the distribution of dominant mtDNA types in Spain may differ from that in Asia. Further research on European and African isolates, both clinical and environmental, is warranted in order to study the molecular epidemiology of *Sporothrix schenckii* at the global level.

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