THE VALUE OF ELECTROPHORETIC PROTEIN PATTERNS FOR THE STUDY OF NOCARDIA SPECIES

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Abstract — *The protein profiles of different species and strains of Nocardia (N.) were compared by using sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) of cytoplasmic extract. In the present study N. asteroides, N. brasiliensis and N. otitidiscaviarum were examined. It was found that protein profiles (Rf: 0.25, 0.37, 0.41, 0.92, 0.98) were specific for Nocardia genus, and some protein fraction (Rf: 1.1) was exclusively found in N. asteroides. A strong similarity was evident between the patterns of N. asteroides and N. brasiliensis, which have a total of five homologous fractions (Rf: 0.08, 0.11, 0.16, 0.51 and 0.63). It seems that SDS-PAGE could be an useful method for the Nocardia typing.*

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INTRODUCTION

Nocardia is gram-positive, partialy acid-fast, branching and filamentous bacteria. The nocardia species most often encountered in human infections are N. asteroides, N. brasiliensis and N. otitidiscaviarum. The clinical findings commonly associated with these organisms are nocardial actinomycetoma and pulmonary and systemic nocardiosis (1). Nocardial actinomycetoma is often caused by direct inoculation of N. brasiliensis or N. otitidiscaviarum into the affected area and pulmonary nocardiosis is caused primarily by N. asteroides (2), with its tendency to disseminate to other organs, offers a poor prognosis for patients.

It has been shown that the serological studies using culture filtrate antigens have demonstrated several N. asteroides serotypes and a species-specific antigen for N. otitidiscaviarum (3,4). Serodiagnosis using the immunodominant proteins found in culture filtrates as antigens has been attempted (1). It has been shown that classification of Nocardia would be helpful in study the epidemiology of nocardiosis in affected animal and human communities as well as in identifying various nocardial strains for studies of pathogenesis, virulence, and ecology. In addition, it has been demonstrated that there are antigenic differences between Nocardia and related genera, also antigenic differences within the genus and within species of Nocardia (3). The aim of present study was to determine whether the protein patterns and composition of the Nocardia are characteristic enough to allow these features to identify the isolates.

MATERIALS AND METHODS

Strains

The isolates examined are listed in Table 1.

Table 1. Source of the Nocardia species studied

Code	Identification	Source
a	N.asteroides (NCPF-1279)	Human pulmonary infection, UK
a_1	N.asteroides	Human cerebral abcess, Iran
a_2	N.asteroides	Human pulmonary infection, Iran
a_3	N.asteroides	Soil, Iran
b	N.brasiliensis (NCPF-1195)	Human mycetoma, Mexico
b_1	N.brasiliensis	Human mycetoma, ---
b_2	N. brasiliensis	Human mycetoma, ---
c	N.otitidiscaviarum (NCPF-1214)	Human mycetoma, UK

NCPF = Culture provided by National Collection of Pathogenic Fungi, Mycological Reference Laboratory, Central Public Health Laboratory, Colindale, London, UK

Cytoplasmic Extract Preparation

Isolates were inoculated for 48 h in three 250 ml flasks with 50 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich) at 37°c in a water bath with constant agitation. The organisms were harvested by centrifugation and washed three times with a sterile saline solution. The pellet was disaggregated at tissue grinder. By this method, the bacterial aggregates were disrupted into individual cells and short filaments that were separated from the large fragments by differential centrifugation as described by Beaman and Maslan (5). To obtain bacterial mass, we inoculated 0.5 ml of this unicellular suspension into five 250 ml flasks each containing 150 ml of brain heart infusion broth. The flasks were incubated at 37°C without agitation for 7 days. The crude extract was obtained by a modified method based on Ortiz-Ortiz and coworkers (6).
The bacterial mass was harvested by centrifugation, washed with warm distilled water, and defatted with ethanol-ethylic ether (1:1). The bacteria were then desiccated under vacuum, and the powder was suspended in 0.01 M Tris-HCl, pH 7.4, with 0.01 M

magnesium acetate. The suspension was sonicated with a 125 W, 20 KHz in a sonicator (Virosonic cell disrupter, Type: KLK 150, Schoellerschall Germany) for 30 minutes in an ice bath set at 2 Sec. cycle, 2 Sec. rest. The sonicated bacteria were centrifuged at 3000g for 15 min to separate fragments and nondisrupted cell. The soluble fraction was obtained by centrifugation at 144,000g for 3 h at 4°C in a L8-70M ultracentrifuge (Beckman, Palo Alto, Calif). The clear supernatant was dialyzed at 4°C for 24 h against distilled water. After dialysis, the solution was lyophilized and stored in aliquots at - 20°C until used (7). The protein content was determined by the Bradford method by using bovine serum albumin (Sigma) as a standard (8).

Polyacrylamide Gel Electrophoresis

0.46 mg freeze-dried extracts were dissolved in 100 μL of doulbe-strength loading buffer (0.125 M Tris-HCl containing 2% sodum dodecyl sulphate, 2% mercapto-ethanol, 10% glycerol, 0.002% bromphenol blue). The samples were heated for 2 minutes and 10 μL containing approximately 3 μg proetin were electrophoresed in 10% polyacrylamide gels with 4% stacking gels. The method was based on that described by Laemmli (9) and Zaini and co - workers (10) with a stocking gel buffer of 0.125 M Tris-HCl pH 6.8, a separating gel buffer of 0.375M Tris-HCl pH 8.8 and a tank running buffer of 0.025 Mm Tris, 0.192 M glycine pH 8.3. Separation was allowed to take place at constant current of 150 V until the tracking dye had run 100 mm. Molecular weight standards were included to facilitate comparison of bands obtained. The standards were from Pharmacia and included Phosphorylase b (94000), bovine serum albumin (67,000) ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and alpha-lactalbumin (14,400). Gels were stained with coomassie blue R-250. After 20 minutes staining, gels were destained with 50% methanol, 10% acetic acid followed by 5% methanol, 10% acetic acid in water until complete clearing of the background had occured. The patterns were scanned by using a densitometer (Helena, process-24) fitted with a 590 nm filter. The molecular weight of proteins were calculated by determining their relative mobilities in 10% polyacrylamide gel and comparing them with those of the molecular weight standards.

RESULTS

A characteristic electrophoretic pattern of distinct protein fractions was obtained from each of the bacterial extracts. Differences were observed among the preparations from the various species in the electrophoretic mobilities of the fractions.

The cytoplasmic extracts from isolates yielded 11-16 fractions. The similarities and differences among the patterns are summarized in Figure 1. Five homologous fractions (Rf: 0.08, 0.11, 0.16, 0.51 and 0.63) were found in N. asteroides and N. brasiliensis. One homologous fraction (Rf: 0.32) was existed in both N. brasiliensis and N. otitidiscaviarum.

The specific fraction (Rf: 1.1) was exclusively found in N. asteroides. Five homologous fractions (Rf: 0.25, 0.37, 0.41, 0.92 and 0.98) also were obtained in all three Nnocardia species.

Densitometer traces of protein patterns of isolates showed an overall similarity between species and strains of species (Figs. 2,3,4).

DISCUSSION

Comparison of protein patterns may be regarded as an indirect method for comparison of microbial DNA, differences in the genome being reflected in the structure of the encoded macromolecules. Good correlation has been found between the results of DNA-DNA hybridization and polyacrylamide gel electrophoresis in a number of bacterial genera (11). The densitometric recording of total protein patterns shows the overall similarity between different isolates and has been used successfully in taxonomic studies of various bacteria (11,12,13). SDS-PAGE was shown to be an effective method for the fractionation and recovery of antigens for serologic tests from the cytoplasmic extracts of Nocardia (3). In this investigation, protein patterns of three species of Nocardia were obtained, and a strong similarity was evident between the patterns of Nocardia species. The homologous fractions (Rf: 0.25, 0.37, 0.41, 0.92, 0.98) were specific for Nocardia genus, and some protein fraction (Rf: 1.1) was exclusively found in N. asteroides, whereas this was not observed in N. brasiliensis or N.otitidiscaviarum. The N. asteroides isolates from patients (a, a_1, a_2) were differentiated from soil isolate (a_3) by fraction (Rf:0.51). Different species were clearly distinguished and within the species, different protein profiles obtained, allowing discrimination of strains. Hopefully, this scheme could promote additional information regarding epidemiologic and ecologic distribution of various strains of Nocardia species. Moreover, such a survey may also have relevance to toxonomic placement of related actionomycetes as suggested by Pier and Fichtner (3). Further studies are needed to asses the utility of this method for detection of immunodominant antigens for use in serologic test.

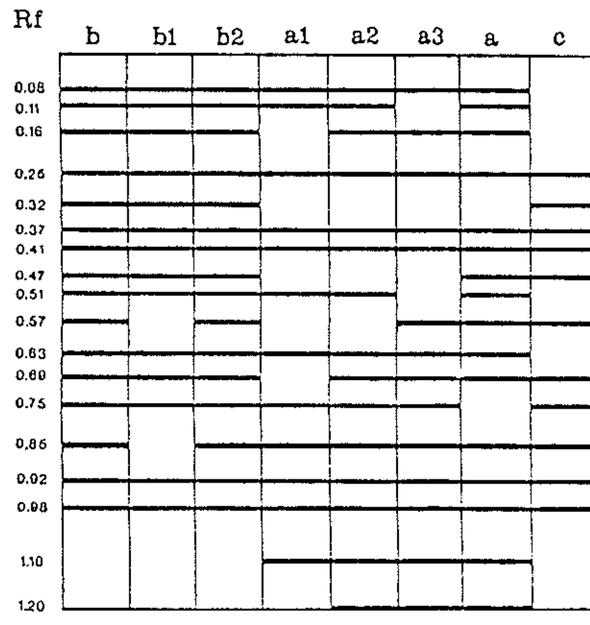

Fig. 1. Diagrammatic comparison of presence or absence of protein fractions observed N. brasiliensis (b_1.b_2.b) ; N. asteroides (a_1.a_2.a_3.a) ; N. otitidiscaviarum (c)

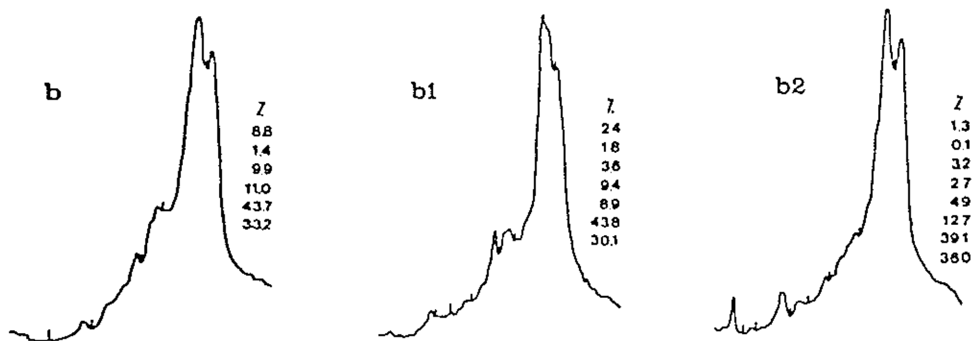

Fig. 2. Densitometer traces of protein patterns of N. brasiliensis

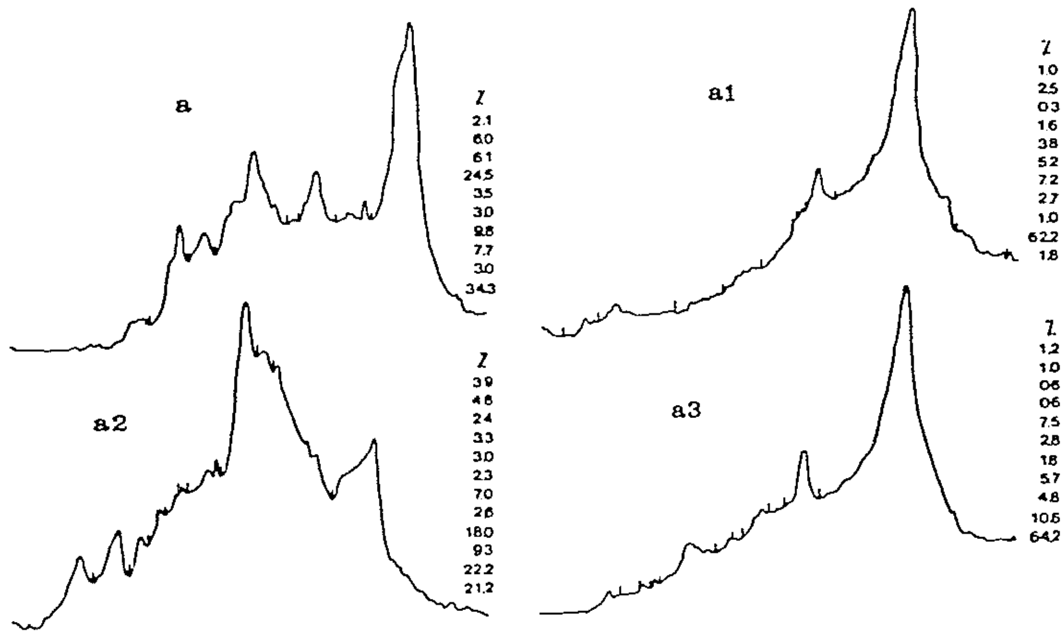

Fig. 3. Densitometer traces of protein patterns of N. asteroides

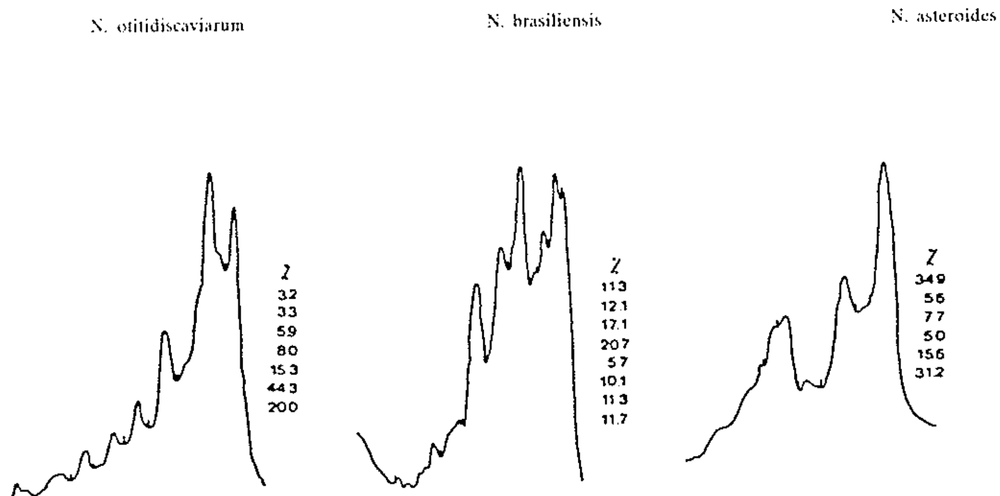

Fig. 4. Densitometer traces of protein patterns of Nocardia species

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