Serotaxonomical Analyses of Some *Streptomyces* and Related Organisms

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Fifteen strains of *Streptomyces*, two of *Streptoverticillium* and one of '*Nocardia*' *mediterranea* were studied by the immunodiffusion technique and the results were compared with those of a previous phenetic study. Both the serological and phenetic analyses demonstrated that '*Nocardia*' *mediterranea* was different from strains of *Streptomyces* and *Streptoverticillium*. The latter two genera were phenetically distinguishable but demonstrated a comparatively close serological relationship. Those strains of *Streptomyces* and *Streptoverticillium* which shared a high number of precipitinogens also belonged to the same phenetic cluster, while strains which were less serologically related usually fell into different clusters. Thus there was good evidence of a general congruence between serological and phenetic similarity. The immunological results also supported the phenetic evidence in suggesting that many species in the genus *Streptomyces* should be combined. Similar evidence was obtained for the genus *Streptoverticillium*.

INTRODUCTION

Immunology has aided the taxonomy of several actinomycete genera, including *Actinomyces* (Holmberg *et al.*, 1975; Bowden *et al.*, 1976), *Mycobacterium*, *Nocardia* and *Rhodococcus* (Ridell & Norlin, 1973; Ridell, 1974, 1975, 1981*a, b*, 1983), *Micropolyspora* (Arden Jones *et al.*, 1979) and *Frankia* (Baker *et al.*, 1981).

The application of immunodiffusion techniques to streptomycetes by Cross & Spooner (1963) demonstrated that serology was of potential taxonomic value in this genus. However, there have been few meaningful serotaxonomic studies subsequently. The results of Kwapinski (1970, 1972) provided a complex pattern of cross-reactions which were of little taxonomic value; the serology of streptomycetes associated with potato scab showed little correlation with their pathogenicity (Bowman & Weinhold, 1963; Vrugink & Maat, 1968).

The serological study of actinomycetes has often been confused by poor selection of strains (Lacey *et al.*, 1978), accentuated by lack of objective classification schemes. Such problems have been particularly marked in the genus *Streptomyces*. However, a recent numerical classification of most of the described species of *Streptomyces* and related genera (Williams *et al.*, 1983) indicated that the number of species could be drastically reduced. It was therefore decided to determine the serological relationships of strains representing some of the major clusters defined by Williams *et al.* (1983) in order to evaluate the integrity of the phenetic groups and the potential of serology in the taxonomy of streptomycetes.

METHODS

Test strains. Fifteen strains of *Streptomyces*, two of *Streptoverticillium* and one of '*Nocardia*' *mediterranea* were studied (Table 1). These represented eleven of the major clusters defined by Williams *et al.* (1983).

Antigen preparation. Strains were inoculated into flasks of glucose (1%, w/v)-nutrient broth (Difco) which were incubated on a rotary shaker at 33 °C. When good growth was obtained, the cells were harvested by filtration, washed three times in 0.01 M-phosphate buffer (pH 7.2) and disintegrated with an X-press apparatus (Edebo,

1960). Cell debris were sedimented by centrifugation (23 500 g for 15 min) and the supernatant extract was diluted in phosphate buffer to give a concentration corresponding to 140 mg semi-dry bacterial mass ml⁻¹. The extracts were then sterilized by filtration.

Antisera preparation. The strains *Streptomyces griseus* ISP 5236, *S. diastaticus* ISP 5496, *S. goshikiensis* ISP 5190 and *Streptovercillium cinnamoneum* ISP 5005 were used for antisera production. These represented the major phenetic clusters 1, 19, 61 and 55, respectively. Rabbits were injected intracutaneously at eight different dorsal sites, with reinjections 21 and 35 d after the initial one. If necessary, additional injections at 14 d intervals were given until a sufficient antibody response was obtained. The suspension used for the initial injections consisted of 2 ml Freund's adjuvant containing 1 ml antigen preparation, 0.9 ml liquid paraffin wax, 0.1 ml Arlacel A and 20 mg cells of the homologous organism, killed by autoclaving for 15 min, dried and ground in a mortar. The injections on day 21 contained 1 ml Freund's adjuvant. The injections on day 35 and any subsequent ones, consisted of 0.5 ml antigen preparation and 0.5 ml 0.01 M-phosphate buffer (pH 7.2) but no adjuvant. The rabbits were bled for control immediately before the first injection and, in order to evaluate the antibody response, one week after each injection. The final bleeding took place two weeks after the last injection.

Immunodiffusion technique. The analyses were carried out using a microplate modification (Wadsworth, 1962) of the immunodiffusion technique of Ouchterlony (1958, 1962).

Reference precipitation systems. Four systems were utilized, one for each of the strains selected for antiserum production. The principles for the establishment and use of serological reference systems have been described previously (Ridell, 1975; Baker *et al.*, 1979). Only the precipitinogens which could be identified by the reference systems were used in the comparison with the phenetic data. However, the occurrence of unidentified precipitinogens was also noted.

RESULTS

The numbers of precipitates identified for the reference systems were 10 for *Streptomyces griseus*, 9 for *S. goshikiensis*, and 8 each for *S. diastaticus* and *Streptovercillium cinnamoneum*. The numbers of precipitinogens shared by each reference and test strain, together with their phenetic similarities (Williams *et al.*, 1983) are given in Table 1. The unidentified precipitates, ranging from one to three, which occurred in most of the test combinations, are not included in these data. Most test strains shared at least one identifiable precipitinogen with the four reference strains. The notable exception was '*Nocardia*' *mediterranea*, which did not form identifiable precipitates with any of the reference sera. Two unidentified precipitates were, however, detected when this strain was analysed by the *S. griseus* system. Likewise, no identifiable precipitates were detected in the *Stv. griseocarneum*-*S. diastaticus* test-combination, but here also some unidentified precipitates occurred.

The *S. griseus* reference strain had the highest number of precipitinogens (6 or 7) in common with strains of its own phenetic sub-cluster (1B). It also had a moderate number of precipitinogens in common with strains from other clusters (1C, 5, 10, 12) with which it shared a similarity of over 74%. However, *S. coelicolor* (sub-cluster 1A) which shared a phenetic similarity with *S. griseus* of 79.2%, produced only two precipitinogens.

Although the *S. diastaticus* reference strain shared two or three precipitinogens with most streptomycetes, it was not closely related serologically to any of the test strains. *Streptomyces diastaticus* was the sole representative of cluster 19 and therefore generally had a fairly low phenetic similarity to the other test strains. The phenetic similarity between *S. diastaticus* and *S. olivaceus* was 79.2%, but these organisms had only three precipitinogens in common.

The *S. goshikiensis* reference strain shared six precipitinogens with *S. lavendulae*, the other representative of cluster 61. Otherwise it showed relatively few reactions, reflecting its generally low phenetic similarity to the other test strains.

A similar pattern was given by the *Streptovercillium cinnamoneum* reference system. This strain shared five precipitinogens with *Stv. griseocarneum*, which was placed into the same phenetic cluster, but shared only one, two or three antigens with the other strains tested.

A summary of the relationships between the phenetic and the serological similarities is given in Table 2.

Table 1. Number of precipitinogens identified by comparative immunodiffusion analysis using four reference systems

Strain*	Cluster number†	No. of precipitinogens using reference system‡:			
		<i>S. griseus</i>	<i>S. diastaticus</i>	<i>S. goshikiensis</i>	<i>Stv. cinnamoneum</i>
<i>Streptomyces griseus</i> ISP 5236§	1B	10 (100)	3 (70.6)	2 (72.7)	3 (66.2)
<i>S. griseinus</i> ISP 5047	1B	7 (81.3)	2 (70.6)	2 (72.7)	1 (61.9)
<i>S. griseobrunneus</i> ISP 5066	1B	6 (81.3)	3 (72.0)	3 (69.8)	1 (64.8)
<i>S. globisporus</i> ISP 5199	1B	7 (87.8)	3 (65.5)	2 (69.1)	2 (62.6)
<i>S. coelicolor</i> ISP 5233	1A	2 (79.2)	3 (74.2)	2 (67.7)	2 (68.4)
<i>S. olivaceus</i> ISP 5072	1C	3 (75.6)	3 (79.2)	3 (68.4)	2 (66.2)
<i>S. exfoliatus</i> ISP 5060	5	4 (74.9)	3 (77.0)	2 (70.6)	1 (71.3)
<i>S. fulvissimus</i> ISP 5593	10	4 (75.6)	2 (70.6)	2 (68.4)	1 (70.6)
<i>S. rochei</i> ISP 5231	12	4 (74.2)	3 (70.6)	2 (72.7)	3 (70.6)
<i>S. albus</i> ISP 5313	16	2 (69.1)	2 (69.8)	1 (66.2)	2 (68.4)
<i>S. diastaticus</i> ISP 5496§	19	2 (70.6)	8 (100)	1 (69.1)	1 (67.0)
<i>S. griseoruber</i> ISP 5281	21	2 (62.6)	2 (77.7)	2 (64.1)	1 (61.9)
<i>S. platensis</i> ISP 5041	29	2 (63.4)	2 (68.4)	2 (69.1)	3 (78.5)
<i>S. lavendulae</i> ISP 5069	61	2 (65.5)	2 (60.5)	6 (79.9)	1 (72.0)
<i>S. goshikiensis</i> ISP 5190§	61	1 (72.7)	1 (69.1)	9 (100)	2 (77.7)
<i>Streptoverticillium cinnamoneum</i> ISP 5005§	55	2 (66.2)	1 (67.0)	2 (77.7)	8 (100)
<i>Stv. griseocarneum</i> ISP 5004	55	2 (73.4)	0 (62.6)	2 (76.3)	5 (79.9)
<i>'Nocardia' mediterranea</i> ATCC 13685	53	0 (69.1)	0 (69.8)	0 (66.2)	0 (65.5)

* Sources: ISP, International Streptomyces Project; ATCC, American Type Culture Collection, Rockville, Md., U.S.A.

† Allocation to clusters defined at the 77.5% S_{SM} similarity level from Williams *et al.* (1983). Sub-clusters 1A, 1B and 1C were defined at the 81% S_{SM} similarity level.

‡ The numbers in parentheses are S_{SM} similarity values from Williams *et al.* (1983).

§ Reference strain.

|| Total number of identifiable precipitates in the reference system.

Table 2. Comparison between the phenetic similarities of reference strains and test strains and the number of precipitinogens shared

S_{SM} value of test strain (%)	Mean no. of precipitinogens shared with reference strain*:			
	<i>S. griseus</i> ISP 5236 (10)	<i>S. diastaticus</i> ISP 5496 (8)	<i>S. goshikiensis</i> ISP 5190 (9)	<i>Stv. cinnamoneum</i> ISP 5005 (8)
> 75	5.6	4.0	4.8	4.5
70-74.9	2.6	2.7	2.0	1.5
65-69.9	1.5	1.5	1.8	1.7
< 65	2.0	1.0	2.0	1.3

* The values in parentheses are the numbers of identifiable precipitates in the reference system.

DISCUSSION

The serological results indicate that *'Nocardia' mediterranea* should be separated from *Streptomyces* and *Streptoverticillium*. This is not only in agreement with the numerical phenetic results of Alderson *et al.* (1981) and Williams *et al.* (1983), but also with chemotaxonomical (Thiemann *et al.*, 1969; Kroppenstedt & Kutzner, 1978; Alderson *et al.*, 1981) and phage host-range studies (Wellington & Williams, 1981).

The two test strains of *Streptoverticillium* shared a limited number of antigens with the strains of *Streptomyces*. These two genera were not clearly distinguished serologically, indicating a comparatively close relationship. Although they can be distinguished phenetically (Williams *et al.*, 1983), they share a cell wall chemotype I (Lechevalier & Lechevalier, 1970) and can be infected by the same phages (Wellington & Williams, 1981).

A large number of described *Streptomyces* and *Streptoverticillium* species have been distributed at the 77.5% S_{SM} similarity level into 19 major, 40 minor and 18 single-membered clusters by numerical phenetic analysis (Williams *et al.*, 1983). The major clusters can be regarded as species or species groups; representatives of nine of these clusters were included in the present study. Although serological cross-reactions occurred between all the streptomycete test and reference strains, the largest numbers of precipitinogens were formed between strains from the same cluster. There was also an overall tendency for the number of precipitinogens to match the similarity levels (Table 2). This relationship was not absolute but, in general, strains with a phenetic similarity of 75% or more shared three or more precipitinogens.

Both the phenetic and serological results indicated that the four strains from cluster 1B (*S. griseus*, *S. griseinus*, *S. griseobrunneus* and *S. globisporus*) are very closely related and represent a single species. A similar conclusion can be drawn for the two strains of cluster 61 (*S. goshikiensis* and *S. lavendulae*) and cluster 55 (*Stv. cinnamoneum* and *Stv. griseocarneum*).

The results of this limited serological study support those of the phenetic classification (Williams *et al.*, 1983), providing further evidence for the over-classification of *Streptomyces* species. They also indicate that serology has potential for the identification and classification of streptomycetes, thus supporting the conclusions of Cross & Spooner (1963).

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