Serological Analysis of Morphologically and Biochemically Similar *Streptomyces* Species

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Descriptions of morphological and biochemical characteristics of *Streptomyces* species have resulted in groups of isolates which appear to be identical or very closely related. Serological analysis has been used to confirm this relationship in some cases and to demonstrate differences in others.

The descriptions of *Streptomyces* species published by Shirling and Gottlieb (4) in the first study of the International *Streptomyces* Project included the following characteristics: spore chain morphology, spore surface, color of colony, reverse side of colony, color in medium, and carbon utilization (including L-arabinose, sucrose, D-xylose, L-inositol, D-mannitol, D-fructose, rhamnose, raffinose, cellulose, D-glucose as carbon sources). An examination of the above descriptions reveals that some of the strains included in this study have identical, or closely similar, characteristics and are not, therefore, readily distinguished as either different species or types. Several groups of cultures may be recognized on this basis. In considering these groups, the following study was done to test the efficiency of serological methods to detect identity of cultures and differences between cultures as correlated with the morphological and biochemical characteristics previously reported.

**MATERIALS AND METHODS**

**Cultures.** All cultures used were obtained from Elwood Shirling, Program Director of the International *Streptomyces* Project collection, and were included in the first study by that group. Stock cultures were maintained by alternate transfer to tomato paste-oatmeal agar (1) and Hickey and Tresner's cobalt-amidex agar (3) at 2-month intervals. Based on similarity of characteristic descriptions, two groups of organisms were chosen for this study. Those organisms designated as group I are listed in Table 1, and differences in characteristics are shown. All cultures in group I were reported to have 10 or more catenulate aerial spores with spiny surfaces, the spore chain described as "Spira," and aerial mass color in the blue series; all produced melanin; all utilized arabinose, sucrose, xylose, inositol, mannitol, fructose, and glucose; and all failed to use cellulose. Within group I, three species, *S. chartreusis, S. lantanus,* and *S. coerulescens,* are identical in all characteristics.

Organisms designated as group II are listed in Table 2 with differences in characteristics. All cultures in group II were reported to have 10 or more catenulate aerial spores with smooth surfaces, the spore chain described as "Rectus-flexibilis," and aerial mass color in the gray series; melanin was not produced; all utilized arabinose, xylose, rhamnose, and glucose; and all failed to utilize sucrose, inositol, raffinose, and cellulose. No two species in this group are identical in all characteristics.

**Antigen preparation.** Cultures were grown and antigen components were prepared by the methods of Taylor et al. (5).

**Antiserum production.** Cell wall fragments for immunization as reported by Guthrie and Taylor (2) were used as immunizing antigens. Immunization schedules and materials used were those used by Taylor et al. (5).

**Serological procedures.** Double-diffusion tests in agar gel used the procedures previously described (5) to determine the antigenic patterns of the cytoplasmic fractions. Serum adsorption was done by using the washed cell wall fraction recovered after sonic oscillation of cultures. One portion of antiserum was mixed with three portions of washed cell walls and incubated for 3 hr at either room temperature or at 5°C. With these proportions, no variations could be detected because of the incubation temperature. Tubes were then centrifuged, and the supernatant-adsorbed serum was removed. In some tests, serum was adsorbed in this manner two and three times to ensure complete removal of specific antibody.

Each antiserum was adsorbed with heterologous cell wall fragments to detect common antigens and cross reactivity. After adsorption, sera were used in diffusion tests or stored at −20°C until use.

**RESULTS**

In tests of group I organisms and antisera, the first observations were made to determine cross reactivity within the group. These results (Table 3) are expressed in numbers of precipitin bands. In cross-reaction patterns, *S. chartreusis* and *S.
lanatus are identical. These two species are identical in previously reported characteristics as well. S. curacoi, distinguished from the two above by its failure to utilize rhamnose and raffinose, shows a similar pattern of cross-reactions, with a difference only in its homologous reaction. S. coerulescens, indistinguishable from S. chartreusis and S. lanatus in previously reported characteristics, shows more differences in cross-reaction patterns, and S. bicolor and S. coerulescens react with considerably different patterns.

To check the reliability of cross-reaction patterns, all antisera were adsorbed with the cell wall fragments of each species in the group. An illustration of results is shown in Table 4.

From results of this type, the data in Table 5 were calculated to indicate the number of precipitin bands remaining after adsorption with cell wall fragments from each species. By using S. chartreusis and S. lanatus as example, adsorption of S. chartreusis antiserum with cell wall fragments of either S. chartreusis or S. lanatus removes all precipitin bands when this adsorbed antiserum is reacted with either S. chartreusis or S. lanatus cytoplasmic fraction. Adsorption of S. lanatus antiserum with S. lanatus cell wall removes all homologous reactivity of this serum; however, adsorption of S. lanatus antiserum with S. chartreusis cell wall leaves one precipitin band when S. lanatus-adsorbed serum is reacted with its homologous cytoplasmic fraction. It, therefore, follows that there is one potential antigenic difference in the cell walls of these two species.

In considering the S. curacoi and S. bicolor

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**Table 1. Differences in characteristics of group I cultures**

| Species designation and ISP no. | Characteristics | Carbohydrate utilization |
|---------------------------------|-----------------|--------------------------|
|                                 | Substrate mycelium color | Soluble pigment | Rhamnose | Raffinose |
| Streptomyces chartreusis 5085   | Yellow           | Negative              | +         | +         |
| S. lanatus 5090                 | Yellow           | Negative              | +         | +         |
| S. coerulescens 5146            | Yellow           | Negative              | +         | +         |
| S. curacoi 5107                 | Red              | Negative              | -         | -         |
| S. coerulesficus 5144           | Yellow           | Negative              | +         | +         |
| S. bicolor 5140                 | Yellow           | Negative              | +         | +         |

**Table 2. Differences in characteristics of group II cultures**

| Species designation and ISP no. | Characteristics | Carbohydrate utilization |
|---------------------------------|-----------------|--------------------------|
|                                 | Substrate mycelium color | Soluble pigment | Mannitol | Fructose |
| Streptomyces flavovirens 5062   | Green           | Negative              | +         | ±        |
| S. nigrifaciens 5071            | Yellow          | Negative              | +         | ±        |
| S. nitrosporeus 5023            | Yellow          | Positive, color variable| -         | -        |

**Table 3. Cross-reactions between group I species**

| Cytoplasm | No. of precipitin bands with antiserum |
|-----------|--------------------------------------|
| ISP no.   | Organism | 5085 | 5090 | 5107 | 5140 | 5144 | 5146 |
| 5085      | Streptomyces chartreusis | (2)<sup>a</sup> | 3    | 1    | 2    | 2    | 1    |
| 5090      | S. lanatus        | 2    | (3)  | 1    | 2    | 2    | 1    |
| 5107      | S. curacoi        | 2    | 3    | (2)  | 2    | 2    | 1    |
| 5140      | S. bicolor        | 1    | 3    | 1    | (4)  | 1    | 1    |
| 5144      | S. coerulesficus  | 1    | 2    | 2    | 2    | (2)  | 2    |
| 5146      | S. coerulescens   | 2    | 3    | 2    | 3    | 2    | (2)  |

<sup>a</sup> Numbers in parentheses indicate homologous reaction.
reactions shown in Table 5, the same reasoning would indicate a total of four potential antigenic differences in the cell walls of these two species. 

Table 6 is a compilation of the potential antigenic differences in the cell walls of these six species. Such differences are determined by adding the precipitin bands appearing when reciprocally adsorbed sera were tested against the cytoplasm of the strain cell wall used for adsorption.

**Table 7. Serological cross-reactions between species of group II**

| Cytoplasm               | No. of precipitin bands with antisera tested |
|-------------------------|---------------------------------------------|
|                         | ISP no.          | Species     | 5062 | 5023 | 5071 |
| S. chartreusis          |                 | Streptomyces flavovirens (2)³ | 1 | 2 |
| S. nitrosporeus         |                 | S. nitrosporeus (3) | 3 | 2 |
| S. nigrifaciens         |                 | S. nigrifaciens (3) | 2 | 2 |

³ Numbers in parentheses indicate homologous reaction.

**Table 8. Reciprocal adsorption of cell wall antisera: homologous reactions**

| Adsorbed with cell wall of | No. of precipitin bands after adsorption by cell wall antiserum and homologous cytoplasmic antigens |
|----------------------------|--------------------------------------------------------------------------------------------------|
| S. chartreusis             | 0º 1 0 1 1 1 1 1 1 1 1 |
| S. nitrosporeus            | 1 0 1 |
| S. nigrifaciens            | 0 1 0 0 0 0 0 0 0 0 0 |

º 0, No apparent antigenic differences.

**Table 9. Serological differences among species of group I in total number of potentially different cell wall antigens**

| Organism                     | Organism compared with |
|-------------------------------|------------------------|
| S. chartreusis               | 5062 5023 5071          |
| S. nitrosporeus              | + + + + + + + + + + + + |
| S. nigrifaciens              | + + + + + + + + + + + + |
| S. chartreusis               | + + + + + + + + + + + + |
| S. nitrosporeus              | + + + + + + + + + + + + |
| S. nigrifaciens              | + + + + + + + + + + + + |

º 0, No apparent antigenic differences.
Reference to Table 5 shows that, when *S. chartreusis* antiserum is adsorbed by *S. coeruleofuscus* cell wall, one precipitin band remains. When the reverse is tested, one precipitin band remains. Thus the potential antigenic difference between the two strains is two since the cell wall of *S. chartreusis* lacks one antigen present in the cell wall of *S. coeruleofuscus*, and the cell wall of *S. coeruleofuscus* lacks one antigen present in the cell wall of *S. chartreusis*. That one antigen is lacking in each case is detected by the remaining precipitin band.

Consideration of group II species was done in the manner used above for group I; the cross-reaction patterns within the group are shown in Table 7. In these results, there are no closely similar patterns of cross-reactivity.

If one analyzes on the basis of homologous reactions after reciprocal adsorption (Table 8), *S. flavovirens* and *S. nigrifaciens* appear to have identical cell wall antigens and appear to be antigenically different from *S. nitrosporeus*. This conclusion is confirmed by calculation of total potentially different antigens (Table 9).

**DISCUSSION**

Previously reported descriptions based on morphological and biochemical characteristics indicate that *S. chartreusis*, ISP 5085; *S. lanatus*, ISP 5090; and *S. coeruleiscens*, ISP 5146 are identical. Serological analysis by cross-reaction patterns and reciprocal absorption techniques show that *S. chartreusis* differs from each of the other two species by one antigen component of the cell wall, whereas *S. lanatus* and *S. coeruleiscens* differ from the other by two cell wall antigen components.

Within this designated group I, *S. curacoii*, ISP 5107, is distinguished by its failure to utilize either rhamnose or raffinose as a carbon source. Serologically, *S. curacoii* is different from both *S. chartreusis* and *S. lanatus* by, again, one antigen component of the cell wall and from *S. coeruleiscens* by two.

*S. bicolor*, ISP 5140, is distinguished from others in group I by the production of a soluble yellow pigment. *S. bicolor* differs from *S. chartreusis* by only one antigen component but is different from all other species in this group by at least three antigen components.

*S. coeruleofuscus*, ISP 5144, is distinguished in this group by a red substrate mycelium or reverse color. This species differs from *S. coeruleiscens* by only one antigen component and from all others by two or more.

From the above results, it appears that antigenic differences among species correlate more closely with pigmentation than with carbohydrate utilization.

Within the designated group II, *S. flavovirens* and *S. nigrifaciens* appear to differ only by a reverse green modification of the substrate mycelium color in *S. flavovirens* and appear to have no antigen component differences in the cell wall. The description of *S. flavovirens* (4) indicates that the green reverse was not always observed and may not, therefore, be a reliable characteristic difference. *S. nitrosporeus* produces a soluble pigment of variable color and fails to utilize either mannitol or fructose as a carbon source. *S. nitrosporeus* differs from both other species in this group by two antigen components. The definite pigmentation characteristic of this species again correlates well with antigenic differences.

From these results, it is apparent that serological methods are useful to reaffirm apparent similarities and differences of morphological and biochemical characteristics of *Streptomyces* species. It appears also that distinct differences in pigmentation of two species indicate greatest potential differences in antigen components of the cell walls of these species. The use of serological characteristics as confirmatory evidence in *Streptomyces* species descriptions appears to be of considerable value in determining identity or nonidentity of two cultures.

**LITERATURE CITED**

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