An Evaluation of Taxonomic Criteria in Streptomyces on the Basis of Deoxyribonucleic Acid Homology

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SUMMARY

Deoxyribonucleic acid (DNA) homologies of 57 cultures of *Streptomyces*, with emphasis on those within the so-called Griseus group, were studied in relation to other taxonomic features used for *Streptomyces*. The DNA samples from the 25 species of the Griseus group, which commonly exhibited the 11 features characteristic of *S. griseus* ISP 5236, showed homology values ranging from 38 to 104 % with the DNA sample from *S. griseus* ISP 5236. Among them, five species showed approximately 40 % homology value and 19 gave values of 56 % or higher. The DNA homologies of 12 streptomycetes which differed from the cultures of the Griseus group in one or two of the 11 selected features were also determined using *S. griseus* ISP 5236 DNA.

The genomic Griseus group was divided into six subgroups on the basis of the homology values with reference DNAs from *Streptomyces griseus* ISP 5236 and *Actinomyces globisporus* ISP 5199. The DNAs of streptomycetes in the largest subgroup were found to be highly homologous to DNA from *S. californicus* ISP 5058 located in that subgroup. From the data obtained, the suitability of several diagnostic features as taxonomic criteria are discussed.

INTRODUCTION

Since Waksman & Lechevalier (1953) proposed the group-species concept in the grouping of *Streptomyces*, many systems for differentiating the groups or species which include the taxon *Streptomyces griseus* have been proposed by Hesseltine, Benedict & Pridham (1954), Baldacci (1958), Mayama (1959), Flaig & Kutzner (1960), Nomi (1960), Baldacci, Locci, Farina & Vegetti (1963), Pridham (1958, 1964), Pridham & Lyons (1965), Hütter (1967), and Gordon & Horan (1968). The criteria in their systems are mainly based on personal experience and not on any phylogenetic evidence. It has not been determined whether phenetic similarity shown by the few characteristics accurately reflects the degree of genetic relatedness (De Ley & Friedman, 1964). Genetic knowledge about bacteria is accumulating in support of the concept of a 'genetic species' or 'genospecies' (Ravin, 1963), which is opposed to the current practice of splitting genera into many species on the basis of only small phenotypic differences (Friedman & De Ley, 1965).

Recently, a series of studies evaluating the reproducibility of taxonomic criteria in *Streptomyces* was undertaken co-operatively under the International Streptomyces Project (Gottlieb & Shirling, 1967). Three hundred cultures were examined (Shirling & Gottlieb, 1968 *a, b*; 1969) on standardized media by means of the criteria and methods described by Shirling & Gottlieb (1966). Fairly high reproducibility of observations of the selected features was obtained with almost all of the cultures tested. However, no attempt has yet been made to combine or

Table 1. *Organisms and the sources, except for streptomycetes identified by their ISP number*

Organism	Source
<i>Streptomyces bikiniensis</i> At-154	IMRU 3514
<i>S. griseinus</i> At-14	NIHJ O-60
<i>S. griseinus</i> At-15	NIHJ O-18
<i>S. griseus</i> At-1	NIHJ S-1
<i>S. griseus</i> At-2	NIHJ S-2
<i>S. griseus</i> At-437	ATU
<i>S. griseus</i> var. <i>rhodochrous</i> At-17	NIHJ SM-2
<i>S. griseus</i> var. <i>rhodochrous</i> At-20	NIHJ SN-14
<i>S. olivaceus</i> At-111	NRRL B-1125
<i>S. olivaceus</i> KCC-67	MTHU 27
<i>S. rameus</i> At-363	NIHJ 1616-Z3

IMRU: Institute of Microbiology, Rutgers State University, U.S.A.

NIHJ: Department of Antibiotics, National Institute of Health, Japan.

ATU: Department of Agricultural Chemistry, University of Tokyo, Japan.

NRRL: Northern Regional Research Laboratory, ARS Culture Collection and Investigations, U.S.A.

MTHU: Department of Bacteriology, Tohoku University, Japan.

divide species in that study. The DNA homologies among actinomycetes have been reported by several researchers (Yamaguchi, 1967; Monson, Bradley, Enquist & Cruces, 1969; Farina & Bradley, 1970) and the methods used were discussed in a previous paper (Okanishi & Gregory, 1970).

In the present study, DNA homologies were used to study the Griseus group of *Streptomyces*, and the traditional criteria for identifying the Griseus group or the species in it were evaluated on the basis of the DNA homology values determined.

METHODS

Fifty-seven streptomycete cultures and a reference strain of *Sarcina lutea* were used. They included 46 cultures, designated by ISP numbers, obtained from the collection of the International Streptomyces Project through the Institute for Fermentation, Osaka (Japan). The features of these streptomycetes described in this paper were taken from the reports of the International Streptomyces Project (Shirling & Gottlieb, 1968*a, b*; 1969). The designations and sources of the other 11 cultures are listed in Table 1.

All streptomycetes were incubated at 27 °C in a minimal medium containing 1.5 % (w/v) glucose, 0.25 % (w/v) asparagine (or 0.3 % (w/v) glycine), 0.1 % (w/v) NH_4NO_3 , 0.05 % (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 % (w/v) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.2 % (v/v) trace element solution (Okanishi & Gregory, 1970), 0.2 % (w/v) KH_2PO_4 , and 0.4 % (w/v) $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$. *Sarcina lutea* was incubated at 27 °C in a Difco nutrient broth supplemented with 0.5 % (w/v) NaCl and 0.1 % (w/v) glucose. Labelling of DNA with radioisotopes (^3H and ^{14}C), and extraction and purification of DNA were carried out as previously described (Okanishi & Gregory, 1970), except that the mycelia used for DNA extraction were incubated with pronase in the solution of 0.4 % (w/v) sodium dodecyl sulphate at 37 °C for 1 h after the lysozyme treatment. The guanine-plus-cytosine content of each DNA preparation was determined by the depurination method of Huang & Rosenberg (1966).

DNA-DNA hybridization was performed by the membrane-filter technique established previously (Okanishi & Gregory, 1970), with 30 μg of sheared-denatured ^{14}C -labelled DNA (SD-DNA) and 2 μg of immobilized ^3H -DNA on a filter (IM-DNA). A filter bearing IM-

DNA was incubated at 48 °C for 20 h in a scintillation vial which contained 0.25 ml of $23\times$ normal strength SSC (SSC is 0.15 M-NaCl plus 0.015 M-sodium citrate), 0.5 ml of formamide, and 0.25 ml of SD-DNA (120 µg/ml). In a previous experiment (Okanishi & Gregory, 1970), maximal hybridization required 40 h incubation but in the present experiment it was attained after 20 h incubation by using a vibrating incubator. At the end of the incubation period, each side of the membrane was washed with 30 ml of double strength SSC in a conical sintered-glass filter by suction filtration. The filters were dried under an infrared lamp for 3 h and then ^3H - and ^{14}C -radioactivities were counted in the two channels of a liquid scintillation counter (LS-200 B, Beckman Instruments, Inc., California, U.S.A.). The homology index of DNA was calculated from the amount of SD-DNA hybridized per unit of IM-DNA retained on a filter. Except for the determinations in Tables 2 and 3, the hybridizations were carried out with duplicate samples, and parts of the determinations were repeated twice. Results were expressed as an average of the determined values, and the values of standard deviation of each determination were lower than 3.0.

RESULTS AND DISCUSSION

Reliability of the determination of DNA homology. The reliability was studied by determining the homologies of DNA from various isolates which were considered to belong to a single species on the basis of traditional taxonomic criteria and the identity of the antibiotic produced. The DNA sample derived from *Streptomyces griseus* ISP 5236 was used as SD-DNA in this hybridization. The DNA homologies in streptomycin-producing *S. griseus* isolates ranged from 98 to 105 %; those of streptomycin-producing *S. griseus* var. *rhodochrous* cultures which were characterized by reddish purple vegetative mycelia were 77 and 81 %; those of grisein-producing *S. griseinus* strains which differed from *S. griseus* in the utilization of L-arabinose and rhamnose ranged from 66 to 71 % (Table 2). Table 2 also shows that if the homology value is calculated on the amount of SD-DNA hybridized, as most investigators have done in order to express the relative homology, errors result from variations in the amount of IM-DNA leached from the filters. These errors could be reduced by determining the amount of IM-DNA retained after the hybridization procedure and by calculating the amount of SD-DNA hybridized per unit of IM-DNA retained. It is apparent that the isolates identified as belonging to a single species have highly homologous DNA, indicating that the assay technique employed is reliable.

Furthermore, DNA-DNA hybridization was studied between *Streptomyces griseus* ISP 5236 and nine streptomycetes distinctly different from *S. griseus*. The majority of these streptomycetes, including species producing antibiotics of the streptomycin group, showed only approximately 40 % homology with the test culture (Table 3). A DNA sample from *Sarcina lutea*, which has the same guanine-plus-cytosine content as the DNA of streptomycetes, showed only 5 % homology. These facts suggest that 40 % of DNA in an individual organism may be common to all species within the genus *Streptomyces*.

DNA homology of streptomycetes related to Streptomyces griseus and traditional taxonomic criteria. Twenty-five streptomycetes were selected from the species described in the International Streptomyces Project by means of the following taxonomic criteria which characterize *Streptomyces griseus*: spore-chain morphology is rectiflexibilis, spore surface is smooth; colour of aerial mycelium belongs to the yellow or red section; melanoid pigments are produced in neither peptone-yeast iron agar nor tryptone-yeast broth; D-glucose, D-xylose, D-mannitol, and D-fructose are utilized for growth; sucrose, *i*-inositol, and raffinose are not utilized. The streptomycetes which were identical in these 11 properties characteristic of

Table 2. DNA-DNA hybridization of isolates classified as *Streptomyces griseus* and *S. griseinus* with *S. griseus* ISP 5236

Source of IM-DNA	(A) IM-DNA ^a retained (μ g)	(B) SD-DNA ^b hybridized (μ g)	(C) B/A $\times$ 100	Homology index
SM ^c -producing <i>Streptomyces griseus</i>				
ISP 5236	1.674	1.252 (100) ^e	74.8	100
ISP 5200 ^d	1.383	1.012 (81)	73.2	98
At-1	1.450	1.104 (88)	76.1	102
At-2	1.644	1.270 (96)	77.3	103
At-437	1.575	1.238 (99)	78.6	105
SM-producing <i>S. griseus</i> var. <i>rhodochrous</i> , VM ^f (reddish purple)				
At-17	1.577	0.906 (72)	57.5	77
At-20	1.571	0.946 (76)	60.2	81
Grisein-producing <i>S. griseinus</i> , Ara ^g (+), Rha ^h (+)				
ISP 5047	1.438	0.713 (57)	49.6	66
At-14	1.144	0.613 (49)	53.6	71
At-15	1.590	0.787 (63)	49.5	66

^aIM-DNA: immobilized ³H-DNA on a filter. ^bSD-DNA: sheared-denatured ¹⁴C-DNA. ^cSM: streptomycin. ^d*Actinomyces streptomycini* Krasil'nikov. ^eRelative amount of the reassociated SD-DNA against that in the homologous hybridization is given in parenthesis. ^fVM: vegetative mycelium. ^gAra: utilization of L-arabinose. ^hRha: utilization of rhamnose.

Table 3. Homology between DNA from streptomycetes clearly different from *Streptomyces griseus* and DNA from *S. griseus* ISP 5236

Source of IM-DNA	(A) IM-DNA retained (μ g)	(B) SD-DNA hybridized (μ g)	(C) B/A $\times$ 100	Homology index
<i>Streptomyces griseus</i> ISP 5236 ^a	1.674	1.252	74.8	100
<i>S. olivaceus</i> ISP 5072	0.858	0.315	36.7	49
<i>S. olivaceus</i> At-111	0.916	0.343	37.4	50
<i>S. olivaceus</i> KCC-67 ^a	1.332	0.418	31.4	42
<i>S. bikiniensis</i> At-154 ^a	1.222	0.512	41.9	56
<i>S. humidus</i> ISP 5263 ^a	1.222	0.357	29.2	39
<i>S. rameus</i> At-363 ^a	1.251	0.347	27.7	37
<i>S. platensis</i> ISP 5041	1.530	0.459	30.0	40
<i>S. venezuelae</i> ISP 5230	1.597	0.464	29.1	39
<i>Streptoverticillium</i> <i>griseocarneum</i> ISP 5004 ^a	1.191	0.356	29.9	40
<i>Sarcina lutea</i>	1.254	0.047	3.75	5

^a Streptomycetes producing streptomycins.

S. griseus were used as a Griseus group in our experiments. Colour of vegetative mycelium, presence or absence of soluble pigments, and utilization of L-arabinose and rhamnose, were not taken as criteria for selecting the Griseus group because *S. griseus* var. *rhodochrous* and *S. griseinus*, which were different from *S. griseus* in the above properties, had provided fairly high homologies with the DNA of *S. griseus* ISP 5236 (Table 2).

Hybridization was attempted using DNA samples from the streptomycetes of the Griseus group and the DNA from *Streptomyces griseus* ISP 5236. As shown in Table 4, the DNA homology values of the 25 designated species ranged from 38 to 104 %. Among them, five species showed approximately 40 % homology value and 19 gave values of 56 % or higher.

Table 4. Homology between DNA from streptomycetes of the *Griseus* group and DNA from *Streptomyces griseus* ISP 5236

Source of IM-DNA	G + C ^a (%)	Homology index
<i>Streptomyces griseus</i> 5236	71	100
<i>S. bikiniensis</i> 5235	73	104
<i>Actinomyces citreofluorescens</i> 5265	72	101
<i>S. ornatus</i> 5307	74	92
<i>S. lipmanii</i> 5070	70	90
<i>S. chrysomallus</i> 5128	72	86
<i>A. vulgaris</i> 5201	71	84
<i>A. fluorescens</i> 5203	73	70
<i>A. globisporus</i> 5199	73	70
<i>S. fimicarius</i> 5322	74	68
<i>A. badius</i> 5139	70	68
<i>S. californicus</i> 5058	70	67
<i>S. pluricologrescens</i> 5019	72	67
<i>S. puniceus</i> 5083	71	64
<i>S. vinaceus</i> 5257	72	64
<i>S. albidus</i> 5320	69	64
<i>A. levoris</i> 5202	ND ^b	63
<i>S. albobiridis</i> 5326	68	60
<i>S. microflavus</i> 5331	ND	60
<i>A. rubiginosohelvolus</i> 5176	69	56
<i>S. felleus</i> 5130	72	51
<i>S. gougeroti</i> 5324	71	43
<i>S. intermedius</i> 5372	71	42
<i>S. sampsonii</i> 5394	ND	42
<i>S. coelicolor</i> 5233	73	42
<i>S. rutgersensis</i> 5077	73	38

^aG + C: guanine-plus-cytosine content.^bND: not done.Table 5. DNA homology between *Streptomyces griseus* ISP 5236 and streptomycetes differing from *S. griseus* in one or two of the features used to define the *Griseus* group

	Source of IM-DNA	Features different from <i>Streptomyces griseus</i>	G + C (%)	Homology index
	<i>Streptomyces griseus</i> 5236		71	100
I	<i>Actinomyces daghestanicus</i> 5149	SC ^a (RA ^b , S ^c)	73	47
	<i>S. griseoviridis</i> 5229	SC(S)	67	58
	<i>S. roseochromogenus</i> 5463	SC(RF ^d , S), SS ^e (spiny)	70	58
II	<i>S. nigrifaciens</i> 5071	AM ^f (grey)	68	43
	<i>S. scabies</i> 5078	AM(grey)	70	42
	<i>S. albidoflavus</i> 5455	AM(white or grey)	70	40
III	<i>A. cyaneofuscatus</i> 5148	MP ^g (+)	71	61
	<i>S. cavourensis</i> 5300	MP(+)	72	67
	<i>S. griseobrunneus</i> 5066	MP(+)	72	64
IV	<i>S. somaliensis</i> 5267	Suc ^h (+)	73	40
	<i>S. cellulosae</i> 5362	Inl ⁱ (+), Suc(+)	71	40
	<i>S. roseolus</i> 5174	Mtl ^j (-)	71	49

^aSC: spore chain morphology. ^bRA: retinaculiaperti. ^cS: spirales. ^dRF: rectiflexibiles. ^eSS: spore surface. ^fAM: aerial mycelium. ^gMP: formation of melanoid pigments. ^hSuc: utilization of sucrose. ⁱInl: utilization of *i*-inositol. ^jMtl: utilization of D-mannitol.

Fig. 1. Diagrammatic grouping of streptomycetes in the Griseus group on the basis of DNA homologies obtained with reference DNAs from *Streptomyces griseus* ISP 5236 and *Actinomyces globisporus* ISP 5199. Each number in a circle identifies an individual type culture. The following list shows the designated species divided into the respective subgroups and indicates the properties of each culture which are different from those of *S. griseus*. Abbreviations used are: AM, aerial mycelium; VM, vegetative mycelium; SP, soluble pigments; Ara, utilization of L-arabinose; Rha, utilization of rhamnose; AB, type of antibiotic produced.

Subgroup 1

1. *Streptomyces griseus* ISP 5236
2. *S. bikiniensis* ISP 5235
3. *Actinomyces citreofluorescens* ISP 5265: SP (yellow or green), Ara(+), Rha(+), AB (actinomycin C?)

Subgroup 2

4. *Streptomyces ornatus* ISP 5307: SP (faint yellow), AB (ornamycin)
5. *S. lipmanii* ISP 5070: VM (yellow to yellow brown), Rha(+)
6. *S. chrysomallus* ISP 5128: SP (yellow), Ara(+), Rha(+), AB (actinomycin C)
7. *S. griseus* var. *rhodochrous* At-20: VM (reddish purple)

Subgroup 3

8. *Actinomyces vulgaris* ISP 5201: Ara(+), Rha(+), AB (pneumocin)

Subgroup 4

9. *Actinomyces globisporus* ISP 5199: SP (yellow to greenish yellow), Ara(+), Rha(+)

Subgroup 5

10. *Streptomyces pluricolorscens* ISP 5019: AM (yellow or red), VM (yellow brown), Rha(+), AB (pluramycin)
11. *Actinomyces badius* ISP 5139: Ara(+)
12. *S. griseinus* ISP 5047: Ara(+), Rha(+), AB (grisein)

The Griseus group used in our experiments was delineated by 11 common features and is more limited than the Griseus group described by other investigators. The range of the DNA homologies of the streptomycetes in this Griseus group, however, is too large to indicate that these cultures belong to a single species. A low homology value of approximately 40 %, which is equivalent to the DNA homology observed between the distinctly different species in the *Streptomyces*, was found in five of the 25 designated species. Generally, when DNA homologies and phenetic similarities are compared, agreement is good among strains of the same taxospecies (Jones & Sneath, 1970). The results obtained indicate that the 11 properties are insufficient criteria for identifying the Griseus group, which is consistent with the DNA homology. Therefore, a group of streptomycetes having at least 56 % homology with the DNA sample from *S. griseus* ISP 5236 was tentatively defined as the genomic Griseus group. The wide range of DNA homology values within this genomic Griseus group indicates that it may still consist of a mixture of different species.

The determination of DNA homologies was expanded to other streptomycetes which differ from *Streptomyces griseus* in one or two of the properties taken to define the Griseus group. DNA-DNA hybridization was tested between the DNA samples from these streptomycetes and the DNA from *S. griseus* ISP 5236. The DNA homologies of the melanoid pigment-producing streptomycetes (group III) ranged from 61 to 67 % with *S. griseus* DNA (Table 5). All streptomycetes forming grey aerial mycelium (group II) and those differing from *S. griseus* in the utilization of D-mannitol, *D*-inositol, or sucrose (group IV) showed approximately 40 % homology. Morphologically different streptomycetes (group I) resulted in intermediate homology values. These data suggest that the lack of formation of melanoid pigments would be of less value than the other properties in identifying the genomic Griseus group. The colour of aerial mycelium and utilization of three kinds of sugars, on the contrary, might be essential criteria for identification of the genomic Griseus group.

Subdivision of streptomycetes related to Streptomyces griseus. Theoretically, the DNA homology obtained by hybridizing with a single reference DNA is not enough to identify a species. Alternative hybridization was performed between the DNA samples from streptomycetes of the Griseus group and the DNA from *Actinomyces globisporus* ISP 5199, whose

13. *A. fluorescens* ISP 5203: SP (faint yellow), Ara(+), Rha(+), AB (actinomycin?)
14. *S. puniceus* ISP 5083: VM (violet), SP (faint blue or violet), AB (viomycin)
15. *S. californicus* ISP 5058: AM (red), VM (violet or red), SP (violet or red), AB (viomycin)
16. *S. vinaceus* ISP 5257: AM (red), VM (reddish purple), SP (red or yellow), AB (viomycin)
17. *S. cavourensis* ISP 5300: MP(+), VM (brown), Ara(+), AB (flavensomycin)
18. *S. albidus* ISP 5320: SP (greenish yellow), Rha(+)
19. *S. griseobrunneus* ISP 5066: MP(+)
20. *S. bikiniensis* At-154: very different
21. *A. rubiginosohelvolus* ISP 5176: VM (pale yellow), SP (yellow or greenish yellow), Ara(+), Rha(+)
22. *S. albobiridis* ISP 5326: Rha(+)
23. *S. microflavus* ISP 5331: AM (grey or yellow), Rha(+)

Subgroup 6

24. *Actinomyces levoris* ISP 5202: Ara(+)
25. *Streptomyces fimicarius* ISP 5322: AM (yellow or white), VM (reddish yellow), SP (red), Ara(+), Rha(+)

Miscellaneous group

26. *Streptomyces felleus* ISP 5130: SP (faint yellow), Ara(+), AB (picromycin)
27. *S. venezuelae* ISP 5230: very different
28. *S. gougeroti* ISP 5324: AM (white or yellow)
29. *S. platensis* ISP 5041: very different
30. *S. intermedius* ISP 5372: Ara(+), AB (polyene)

homology with *Streptomyces griseus* DNA was 70 %. Among the 30 designated species tested, the 25 species which showed at least 56 % homology with *S. griseus* DNA showed 59 % or higher homology with *A. globisporus*, with the exception of *S. fimicarius* and *S. levoris* (Fig. 1). *Streptomyces fimicarius* and *S. levoris* showed only 47 and 50 % homology, respectively, with *A. globisporus* DNA but their homology values with *S. griseus* DNA were 68 and 63 %, respectively. The four species which showed approximately 40 % homology with *S. griseus* DNA also showed about 40 % homology with *A. globisporus*. Therefore, it is concluded that the genomic Griseus group may be defined as the group having at least 56 % homology with *S. griseus* ISP 5236 DNA and at least 47 % homology (the majority has at least 59 % homology) with *A. globisporus* ISP 5199 DNA.

The genomic Griseus group was diagrammatically divided into two large and four small subgroups, on the basis of the homology values obtained with the reference DNAs from *Streptomyces griseus* and *Actinomyces globisporus*. The legend to Fig. 1 shows the described species which are divided into subgroups and indicates the features of each species which are different from those of *S. griseus*. The DNA homology values of the three species in the subgroup 1 showed approximately 100 and 80 % with *S. griseus* DNA and *A. globisporus* DNA, respectively. *Actinomyces citreofluorescens* differs from the other two in soluble pigments, utilization of L-arabinose and rhamnose, and type of antibiotic produced (Shirling & Gottlieb, 1968*a, b*). Similar differences within a subgroup were also found in the subgroups 2, 5 and 6.

Further hybridizations were done to confirm that the largest subgroup (subgroup 5 in Fig. 1) consisted of closely related streptomycetes. The DNA sample of *Streptomyces californicus* ISP 5058, located at the centre of this subgroup 5, was used as the SD-DNA. Homology values of 88 % or higher were obtained in 10 of the 13 species tested while the three other species had homology values ranging from 71 to 83 % (Table 6). These data apparently suggest that each subgroup in the genomic Griseus group represents an independent taxon, probably corresponding to a species. Each subgroup, however, consists of the designated species differing from one another in colour of vegetative mycelium, soluble pigments, utilization of L-arabinose and rhamnose, and type of antibiotic produced (legend to Fig. 1). These findings suggest that these features are not suitable criteria for species identification in the genomic Griseus group, but may differentiate strains within a species.

The melanin-forming streptomycetes, such as *Streptomyces cavourensis*, *Actinomyces cyaneofuscatus*, and *S. griseobrunneus*, displayed fairly high homologies (at least 61 %) with *S. griseus* DNA (Table 5) and at least 90 % homology with the DNA from *S. californicus*, a melanin-negative strain (Table 6). The lack of formation of melanoid pigments has been considered to be an important criterion for distinguishing the Griseus group by many researchers, with the notable exception of Pridham (1964) and Pridham & Lyons (1965). However, from the data described above, the lack of melanin formation is not considered to be useful as a criterion for distinguishing the genomic Griseus group or a species within it. This conclusion is supported by the reports of Gregory & Huang (1964*a, b*) that tyrosinase inheritance in *S. scabies* is controlled by a plasmid which can replicate faster than the chromosomal genome. On the other hand, we found that the colour of aerial mycelium and the utilization of three kinds of sugars were useful diagnostic characters for the identification of the genomic Griseus group (Table 5).

Reliability of the DNA homology values determined. The DNA homologies in reciprocal hybridizations and in the viomycin-producing strains were checked in Table 6. The homology (67 %) of *Streptomyces griseus* DNA to *S. californicus* DNA was similar to the homology (67 %) of *S. californicus* DNA to *S. griseus* DNA. Similar results were also obtained for

Table 6. DNA homologies of streptomycetes closely related to *Streptomyces californicus* ISP 5058

Source of IM-DNA	Source of SD-DNA		
	<i>Streptomyces californicus</i> ISP 5058	<i>S. griseus</i> ISP 5236 ^a	<i>Actinomyces globisporus</i> ISP 5199 ^b
<i>Streptomyces californicus</i> 5058	100	67	71
<i>S. vinaceus</i> 5257	98	64	69
<i>S. puniceus</i> 5083	97	64	71
<i>S. bikiniensis</i> At-154	96	56	74
<i>S. griseinus</i> 5047	93	66	75
<i>S. griseobrunneus</i> 5066	91	64	66
<i>S. cavourensis</i> 5300	90	67	69
<i>S. albidus</i> 5320	90	64	67
<i>S. microflavus</i> 5331	90	60	65
<i>Actinomyces badius</i> 5139	89	68	77
<i>S. pluricolorescens</i> 5019	88	67	78
<i>A. fluorescens</i> 5203	83	70	74
<i>S. alboviridis</i> 5326	79	60	65
<i>A. rubiginosohelvolus</i> 5176	71	56	71
<i>S. griseus</i> 5236	60	100	78
<i>A. globisporus</i> 5199	75	70	100

^a Data derived from Tables 2 and 4. ^b Data derived from Fig. 1.

S. californicus–*Actinomyces globisporus* pair, 75 and 71 %, and for *S. griseus*–*A. globisporus* pair, 70 and 78 %. On the other hand, *S. californicus*, *S. vinaceus* and *S. puniceus*, producers of viomycin, were apparently a single taxon, showing at least 97 % homology with *S. californicus* ISP 5058 DNA. Similarity of the DNA homology values in these three strains was also obtained by hybridizing with *S. griseus* ISP 5236 DNA, indicating 67, 64, and 64 %, respectively, and with *A. globisporus* ISP 5199 DNA, indicating 71, 69, and 71 %, respectively. The reasonable agreement of homology values in these reciprocal hybridizations or in the DNAs of viomycin-producing strains by using the three reference DNAs attests to the reliability of the DNA homology values determined.

The DNA homologies of *Streptomyces bikiniensis* At-154 and *S. bikiniensis* ISP 5235 with *S. griseus* DNA were of 56 % (Table 3) and 104 % (Table 4), respectively. Although both strains allegedly originated from the same strain IMRU 3514 (Waksman), the features of ISP 5235 (Shirling & Gottlieb, 1968b) are very different from those given in the original descriptions (Johnston & Waksman, 1948; Pridham & Lyons, 1965) in the colour of aerial mycelium, the pattern of sugar utilization, and the formation of melanoid pigments. The features of At-154, on the other hand, are identical to those in the original descriptions. These facts suggest that, at some time, some error in transferring or labelling *S. bikiniensis* ISP 5235 may have occurred.

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