Morphological Subtype of the Smooth-Spored Streptomycetes

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Received for publication 31 January 1966

ABSTRACT

TRESNER, H. D. (Lederle Laboratories, American Cyanamid Co., Pearl River, N.Y.), M. C. DAVIES, AND M. E. ENGLERT. Morphological subtype of the smooth-spored streptomycetes. *J. Bacteriol.* **91**:1998–2005. 1966.—Electron microscopy reveals that certain *Streptomyces* species produce chains of smooth-surfaced, contiguous, cylindrical spores that resemble the phalanges. Although spore shape itself has not, heretofore, provided an especially important aid in the taxonomy of the streptomycetes, the “phalangiform” spore character promises to be helpful in the identification of species possessing it.

Through the use of electron microscopy in recent years, it has been adequately established that the micromorphology of streptomycete spore surfaces is sufficiently distinctive as to be a useful taxonomic criterion. It is commonly agreed that spore surfaces may be characterized according to four basic types: smooth, warty, spiny, and hairy.

From a study of the spore morphology of some 175 streptomycete “subgroups,” Kutzner (Dissertation, Landwirtschaftliche Hochschule, Hohenheim, Germany, 1956) determined that 133 (76%) produced smooth-spored types. Similarly, in a more recent investigation of a group of 120 *Streptomyces* species, Tresner, Davies, and Backus (6) found that approximately 80% of the species possessed smooth-surfaced spores. The disproportionately large number of species having smooth spores has, therefore, offered a challenge to devise means for subdividing this group into more manageable taxonomic units. Consequently, our attention has been focused on various other morphological details of spores as possible avenues for achieving this objective.

Unlike the situation in many of the higher cryptogamic organisms, differences in spore dimensions of the streptomycetes are not often of special taxonomic significance. Most streptomycete spores fall within a rather limited size range. Moreover, considerable variation in linear dimensions may occur within a single species or even strain. Spore shape, on the other hand, can sometimes provide a more valuable implement for species differentiation, and it is with this aspect that the present work is concerned.

MATERIALS AND METHODS

Electron microscopy. The RCA EMU 3C electron microscope was used in the examination of spore preparations. For the determination of spore shape and surface configuration, a simple spore print technique was used, in which copper grids (200-mesh) coated with polyvinyl formal were pressed gently to the sporulating surface of cultures. Adhering spore chains, without further treatment, were viewed directly in the microscope at about 8,000 magnifications.

Material for sectioning was obtained from strips cut through live sporulating colonies *in situ* on the agar medium. These preparations proved superior to those made from pelleted spore suspensions, because they yielded more spore chains that were intact. The agar strips were fixed for 18 hr in 1% osmium tetroxide at room temperature according to the method of Kellenberger, Ryter, and Séchaud (1). The fixative was removed by rinsing in tryptone medium (Difco tryptone, 1%; NaCl, 0.5%) to which had been added a few drops of dilute detergent (sodium dodecyl sulfate). While in this solution, the strips were cut into 1-mm cubes. After 1 hr of exposure to 0.5% uranyl acetate, the pieces were rapidly dehydrated in a graded ethyl alcohol series and embedded in 80% *n*-butyl methacrylate-20% methyl methacrylate containing 1.5% Luperco CDB (Lucidol Division, Wallace and Tiernan Inc., Buffalo, N.Y.). Blocks were polymerized at 70 C for 18 hr. Sections were cut with a Porter-Blum microtome equipped with a diamond knife, and were deposited on carbon-coated copper grids.

Culture materials. Although a substantial number of the organisms studied were obtained from reference culture collections, in some instances multiple strains of certain species were provided from our collection of

FIG. 1. *Phalangiiform spores of Streptomyces aureofaciens ATCC 10762.*

soil isolates. The cultures were grown in petri dishes for 14 days at 28 C on three agar media [yeast extract-agar (3), tomato paste-oatmeal-agar (3), and oatmeal-agar (2)]. Usually, one or more of these media provided adequate sporulation. Except for the organisms used in spore sectioning, mature cultures were

preserved with formalin (4) and stored at 4 C until needed.

RESULTS AND DISCUSSION

In our studies of large numbers of smooth-spored streptomycetes, we have observed four

FIG. 2. Longitudinal thin section of a spore chain of *Streptomyces aureofaciens* ATCC 10762.

general spore shapes, ranging from subglobose to elliptical to elongate to cylindrical. Although these categories are arbitrary, they generally are not difficult to differentiate. Most confusion is likely to be encountered between the elongate and cylindrical types. Our interpretation of cylindrical spores follows the definition that "geometrically a cylinder is the volume generated by a rectangle rotated about one of its parallel axes." By definition, cylindrical spores, then, have parallel linear walls and are truncate, whereas elongate spores are all other extremely protracted shapes not conforming entirely to this description.

A few minor modifications of the general spore types occur. Also, variability in spore shape may be encountered among different strains of some species, and even within a single strain in particular instances. Usually, the variability does not exceed one gradation, i.e., from globose to elliptical or from elliptical to elongate. Certain of the cylindrical spore types appear to have

considerable stability in form, and offer possibilities as taxonomic aids.

A novel cylindrical type, which we have designated "phalangiform" or "phalangeal," appears to be useful for subdividing the smooth-spored forms. As the name implies, chains of phalangiform spores resemble the phalanges, when viewed in whole mounts by electron microscopy. In Fig. 1, the characteristically phalangiform spores of *S. aureofaciens* are shown. The electron-dense material within the spores presents the appearance of a "dumbbell" or "hourglass" having a thick wall. The end surfaces are flattened where adjoining cells abut. Enveloping the spore chain is a thin, less electron-dense membrane which may or may not be collapsed, depending upon the condition of the preparation.

By use of light microscopy (at 1,000 $\times$), crystal violet-stained whole mounts of phalangiform spores generally reveal a differential staining of those spore regions corresponding to the

TABLE 1. *Smooth-spored streptomycetes*

<i>Streptomyces</i> culture	Pha- langi- form spores present	Source* and/or no.	Spore- color series†	<i>Streptomyces</i> culture	Pha- langi- form spores present	Source* and/or no.	Spore- color series†
<i>S. achromogenes</i> ...		ATCC 12767	GY	<i>S. canescens</i>		NRRL B-2419	Y
<i>S. achromogenes</i>				<i>S. canus</i>		ATCC 12647	GY
var. <i>streptozoti-</i>				<i>S. canus</i>		ATCC 12646	GY
<i>cus</i>		NRRL 2697	GY	<i>S. capreolus</i>		NRRL 2773	Y
<i>S. acidomyceticus</i> ...	X	ATCC 11611	R	<i>S. carneus</i>		ATCC 6847	W
<i>S. actuosus</i>		NRRL 2954	GY	<i>S. carnosus</i>		CBS	GY
<i>S. albidoflavus</i>		NRRL B-1271	Y	<i>S. catenulae</i>		ATCC 12476	GY
<i>S. albidoflavus</i>		CBS	Y	<i>S. cavourensis</i>		NRRL 2740	Y
<i>S. albireticuli</i>		IFO 3400	Y	<i>S. cellulosa</i>		ATCC 3313	Y
<i>S. alhoflavus</i>		CBS	W	<i>S. chrysomallus</i> ...		ATCC 11523	Y
<i>S. alboniger</i>		ATCC 12461	W	<i>S. cinereoruber</i>			
<i>S. albosporeus</i>		ATCC 3003	Y	var. <i>fructofer-</i>			
<i>S. albus</i>		ATCC 3361	Y	<i>mentans</i>		NRRL 2588	GY
<i>S. albus</i>		ATCC 3351	Y	<i>S. cinnamomeus</i> ...		ATCC 11874	R
<i>S. albus</i>		NRRL B-683	Y	<i>S. cinnamomeus</i> f.			
<i>S. albus</i>		ATCC 3004	W	<i>azacoluta</i>		ATCC 12686	R
<i>S. albus</i>		ATCC 3006	Y	<i>S. cinnamonensis</i> ...	X	ATCC 12308	R
<i>S. albus</i>		ATCC 3005	W	<i>S. cinnamonensis</i> ...	X	NRRL B-1588	R
<i>S. albus</i>		ATCC 618	W	<i>S. cinnamonensis</i> ...		ATCC 15413	R
<i>S. albus</i>		CBS	W	<i>S. circulatus</i>		CBS	R
<i>S. albus</i>		NRRL B-2490	GY	<i>S. cirratus</i>		ATCC 14699	GY
<i>S. albus</i>		NRRL B-2401	R	<i>S. citreus</i>		ATCC 10974	Y
<i>S. albus</i> var.				<i>S. citreus</i>		CBS	Y
<i>phathocidicus</i> ...		ATCC 14510	W	<i>S. clavifer</i>		CBS	W
<i>S. ambofaciens</i> ...		ATCC 15154	GY	<i>S. coelicolor</i>		CBS	Y
<i>S. aminophilus</i> ...		ATCC 14961	Y	<i>S. coelicolor</i>		IMRU 3593	Y
<i>S. antibioticus</i> ...		NRRL B-1701	GY	<i>S. coelicolor</i> var.			
<i>S. antibioticus</i> ...		ATCC 10382	GY	<i>aminophilus</i>		NRRL 2390	Y
<i>S. antibioticus</i> ...		ATCC 11891	GY	<i>S. corchorasii</i> ...	X	NCIB 9476	GY
<i>S. antibioticus</i> ...		CBS	GY	<i>S. cremeus</i>		CBS	R
<i>S. antibioticus</i> ...		ATCC 14888	GY	<i>S. cyaneofascatus</i> ...		Gause 99/54	W
<i>S. anulatus</i>		ATCC 3307	Y	<i>S. cyaneogriseus</i> ...		Gause 390	GY
<i>S. ardens</i>	X	NRRL 2817	GY	<i>S. daghestanicus</i> ...		Gause 13897/54	R
<i>S. argenteolus</i> ...		ATCC 11009	GY	<i>S. diastaticus</i>		CBS	Y
<i>S. atrofaciens</i> ...		NRRL 2429	R	<i>S. diastaticus</i>		ATCC 3315	Y
<i>S. aureofaciens</i> ...	X	ATCC 10762	GY	<i>S. diastatochro-</i>			
<i>S. aureofaciens</i> ...	X	NRRL B-1286	GY	<i>mogenes</i>		NRRL 2772	GY
<i>S. aureofaciens</i> ...	X	NRRL B-1287	GY	<i>S. distallicus</i>		NRRL 2886	R
<i>S. aureofaciens</i> ...	X	NRRL B-1288	GY	<i>S. endus</i>	X	NRRL B-2339	GY
<i>S. aureofaciens</i> ...	X	ATCC 12416a	GY	<i>S. endus</i>	X	NRRL 2736	GY
<i>S. aureofaciens</i> ...	X	ATCC 13899	GY	<i>S. erythraeus</i>		ATCC 11912	Y
<i>S. aureofaciens</i> ...	X	ATCC 13900	GY	<i>S. erythraeus</i>		CBS	Y
<i>S. aureus</i>	X	ATCC 3309	GY	<i>S. erythraeus</i>		NCIB 8665	Y
<i>S. aureus</i>		NRRL B-1265	GY	<i>S. eurocidicus</i> ...		NRRL B-1676	Y
<i>S. aureus</i>		ATCC 15437	GY	<i>S. eurythermus</i> ...	X	NRRL 2539	GY
<i>S. badius</i>		CBS	Y	<i>S. eurythermus</i> ...	X	NRRL 2540	GY
<i>S. bikiniensis</i> ...		NRRL B-1049	GY	<i>S. eurythermus</i> ...	X	NRRL 2541	GY
<i>S. bikiniensis</i> ...		ATCC 11062	GY	<i>S. exfoliatus</i>	X	ATCC 12627	R
<i>S. bikiniensis</i> ...		NRRL 2737	GY	<i>S. felleus</i>		NRRL B-2251	GY
<i>S. cacaoi</i>		ATCC 3082	Y	<i>S. fimicarius</i>		CBS	GY
<i>S. cacaoi</i>		CBS	GY	<i>S. flaveolus</i>	X	ATCC 13811	GY
<i>S. caelestis</i>		NRRL 2418	B	<i>S. flavidovirens</i> ...		Gause 12287	Y
<i>S. caeruleus</i>		NRRL B-1623	GY	<i>S. flavidovirens</i>			
<i>S. californicus</i> ...		ATCC 15436	Y	var. <i>fusca</i>		Gause 6786/54	Y
<i>S. canarius</i> var.				<i>S. flavochromo-</i>			
<i>canarius</i>		NRRL 2976	Y	<i>genes</i>		ATCC 14841	GY
<i>S. candidus</i>		ATCC 4878	Y	<i>S. flavogriseus</i> ...		CBS	GY
<i>S. candidus</i>		CBS	W	<i>S. flavotricini</i> ...		Gause 1436	Y
<i>S. canescens</i>		ATCC 15731	Y	<i>S. flavovirens</i> ...		ATCC 3320	GY

TABLE 1—continued

<i>Streptomyces</i> culture	Pha- langi- form spores present	Source* and/or no.	Spore- color series†	<i>Streptomyces</i> culture	Pha- langi- form spores present	Source* and/or no.	Spore- color series†
<i>S. flavus</i>		CBS	W	<i>S. griseus</i> var.			
<i>S. flavus</i>		ATCC 3369	GY	<i>purpureus</i>		ATCC 11861	Y
<i>S. fradiae</i>		ATCC 11903	R	<i>S. griseus</i> var.			
<i>S. fradiae</i>		ATCC 10745	R	<i>purpureus</i>		NRRL 2423	Y
<i>S. fradiae</i>		NCIB 9009	R	<i>S. griseus</i> var.			
<i>S. fradiae</i>		NRRL 2598	R	<i>purpureus</i>		NRRL B-1381	Y
<i>S. fradiae</i>		ATCC 14544	R	<i>S. griseus</i> var.			
<i>S. fradiae</i>		ATCC 15438	R	<i>spiralis</i>		ATCC 13733	Y
<i>S. fragilis</i>		NRRL 2424	R	<i>S. halstedii</i>		CBS	GY
<i>S. fulvissimus</i>		NRRL B-1453	R	<i>S. halstedii</i>		ATCC 10897	GY
<i>S. fumosus</i>		Gause 871	GY	<i>S. hiroshimensis</i> ..		NRRL B-1823	R
<i>S. galbus</i>	X	ATCC 14077	GY	<i>S. hominis</i>		ATCC 3008	Y
<i>S. galilaeus</i>		NRRL 2722	GY	<i>S. humidus</i>	X	ATCC 12760	GY
<i>S. galtieri</i>		CBS	W	<i>S. hygroscopicus</i> ..	X	CBS	GY
<i>S. gelaticus</i>		ATCC 3323	GY	<i>S. hygroscopicus</i> ..	X	NRRL B-1503	GY
<i>S. globisporus</i>		Gause 13024	Y	<i>S. hygroscopicus</i> ..	X	NRRL B-1346	GY
<i>S. globisporus</i> var.				<i>S. hygroscopicus</i> ..	X	ATCC 10976	GY
<i>caucasicus</i>		Gause 13195/54	Y	<i>S. hygroscopicus</i> ..	X	IFO 3401	GY
<i>S. globisporus</i> var.				<i>S. hygroscopicus</i> ..	X	NRRL B-1865	GY
<i>flavofuscus</i>		Gause 1565/53	Y	<i>S. hygroscopicus</i> ..	X	NRRL 2751	GY
<i>S. globisporus</i> -				<i>S. hygroscopicus</i> ..	X	NRRL 2752	GY
<i>griseus</i>		Gause 4246	GY	<i>S. hygroscopicus</i> ..	X	NRRL 2387	GY
<i>S. globosus</i>	X	ATCC 14979	R	<i>S. hygroscopicus</i> ..	X	NRRL 2388	GY
<i>S. gobitricini</i>		Gause 5618	R	<i>S. hygroscopicus</i> ..	X	NRRL 2389	GY
<i>S. gougeroti</i>		ATCC 10975	Y	<i>S. hygroscopicus</i> ..	X	ATCC 13810	GY
<i>S. griseocarneus</i> ..		ATCC 12628	R	<i>S. hygroscopicus</i> ..	X	ATCC 15166	GY
<i>S. griseofaciens</i> ..		ATCC 13180	GY	<i>S. hygroscopicus</i>			
<i>S. griseolus</i>		ATCC 3325	GY	var. <i>decoyinine</i> ..		NRRL 2666	GY
<i>S. griseolus</i>		IFO 3403	GY	<i>S. hygroscopicus</i>			
<i>S. griseoluteus</i> ..		IMRU 3674	R	<i>f. glebosus</i>		ATCC 14607	GY
<i>S. griseoluteus</i> ..	X	ATCC 12768	GY	<i>S. intermedius</i> ..		ATCC 3329	Y
<i>S. griseoroseus</i> ..		ATCC 12125	Y	<i>S. ipomoea</i>		ATCC 11747	GY
<i>S. griseoruber</i>	X	NRRL B-1818	GY	<i>S. jujuy</i>		ATCC 13670	Y
<i>S. griseospiralis</i> ..		NRRL 3018	GY	<i>S. kanamyceticus</i> ..		ATCC 12853	Y
<i>S. griseoviridis</i> ..		NRRL 2427	Y	<i>S. kentuckensis</i> ..		ATCC 12691	R
<i>S. griseoviridis</i>				<i>S. kitasatoensis</i> ..		Hata	Y
var. <i>atrofaciens</i> ..		NRRL 2563	R	<i>S. krestomyceticus</i> ..		ATCC 14947	W
<i>S. griseoviridis</i>				<i>S. kurssanovii</i>		Gause 7069a/53	GY
var. <i>atrofaciens</i> ..		NRRL 2715	R	<i>S. lavendulae</i>	X	ATCC 14158	R
<i>S. griseus</i>		ATCC 13968	Y	<i>S. lavendulae</i>	X	ATCC 8664	R
<i>S. griseus</i>		NRRL B-599	Y	<i>S. lavendulae</i>	X	ATCC 11924	R
<i>S. griseus</i>		ATCC 3326	Y	<i>S. lavendulae</i>	X	NRRL B-547	R
<i>S. griseus</i>		ATCC 10971	Y	<i>S. lavendulae</i>	X	NRRL B-1230	R
<i>S. griseus</i>		ATCC 11746	Y	<i>S. lavendulae</i>	X	NRRL B-1259	R
<i>S. griseus</i>		NRRL B-150	Y	<i>S. lavendulae</i>	X	NRRL B-2402	R
<i>S. griseus</i>		NRRL 2607	Y	<i>S. lavenduligriseus</i>		ATCC 13306	GY
<i>S. griseus</i>		NRRL 2426	Y	<i>S. lincolnensis</i> var.			
<i>S. griseus</i>		ATCC 12475	Y	<i>lincolnensis</i>		NRRL B-2936	R
<i>S. griseus</i>		NRRL 2719	Y	<i>S. lipmanii</i>		ATCC 3331	Y
<i>S. griseus</i>		NRRL 2720	Y	<i>S. lipmanii</i>		CBS	Y
<i>S. griseus</i>		ATCC 15395	Y	<i>S. loidensis</i>		ATCC 11415	GY
<i>S. griseus</i>		ATCC 15569	Y	<i>S. longissimus</i>		ATCC 14562	Y
<i>S. griseus</i> var.				<i>S. lydicus</i>		NRRL 2433	GY
<i>desideus</i>		NRRL 2971	Y	<i>S. mediocidicus</i> ..		ATCC 13278	Y
<i>S. griseus</i> f. <i>fari-</i>				<i>S. mediocidicus</i> ..		ATCC 13279	Y
<i>nosus</i>		ATCC 13741	Y	<i>S. mediterranei</i> ..		ATCC 13685	W
<i>S. griseus</i> var.				<i>S. melanogenes</i> ..	X	ATCC 12851	R
<i>purpureus</i>		ATCC 3312	Y	<i>S. melanogenes</i> ..		ATCC 12852	R

TABLE 1—continued

<i>Streptomyces</i> culture	Pha- langi- form spores present	Source* and/or no.	Spore- color series†	<i>Streptomyces</i> culture	Pha- langi- form spores present	Source* and/or no.	Spore- color series†
<i>S. michiganensis</i> ...		ATCC 14970	Y	<i>S. rochei</i>		ATCC 10739	R
<i>S. microflavus</i>		ATCC 13231	Y	<i>S. roseochromo-</i> ...		CBS	R
<i>S. mirabilis</i>		NRRL B-2400	GY	<i>genus</i>		ATCC 3347	Y
<i>S. misionensis</i>	X	ATCC 14991	GY	<i>S. roseochro-</i> ...		IFO 3363	R
<i>S. murinus</i>		NRRL B-2286	GY	<i>genus</i>	X	IFO 3411	R
<i>S. naganishii</i>		NRRL B-1816	GY	<i>S. roseochromo-</i> ...	X	ATCC 13400	Y
<i>S. naraensis</i>	X	ATCC 13788	GY	<i>genus</i>		NRRL 2294	R
<i>S. narbonensis</i>		CBS	GY	<i>S. roseochromo-</i> ...		NRRL B-1566	R
<i>S. narbonensis</i>		NRRL B-1680	GY	<i>genus</i>		ATCC 13167	R
<i>S. netropsis</i>		NRRL 2268	R	<i>S. roseochromo-</i> ...		ATCC 12414	GY
<i>S. netropsis</i>		ATCC 11874	R	<i>genus</i>		Gause 1450	R
<i>S. nigellus</i>	X	NRRL 2920	GY	<i>S. roseoflavus</i>		NRRL 2776	GY
<i>S. nitrificans</i>		NRRL B-1664	W	<i>S. roseoflavus</i>		Gause 11351	R
<i>S. nitrosporeus</i>		ATCC 12769	GY	<i>S. roseoflavus</i>		NRRL B-1484	R
<i>S. nitrosporeus</i>		IFO 3362	GY	<i>S. roseogriseus</i>		ATCC 12629	R
<i>S. niveus</i>		NRRL 2466	Y	<i>S. roseolilacinus</i> ...			
<i>S. nodosus</i>	X	ATCC 14899	GY	<i>S. roseoluteus</i>			
<i>S. nogalater</i> var.				<i>S. rubiginoso-</i> ...			
<i>nogalater</i>		NRRL 3035	GY	<i>helvolus</i>			
<i>S. odorifera</i>		ATCC 6246	Y	<i>S. rubrircetuli</i>			
<i>S. olivaceus</i>	X	ATCC 11626	GY	<i>S. rubrircetuli</i>			
<i>S. olivochromo-</i> ...				<i>S. rubrocycano-</i> ...			
<i>genes</i>		ATCC 3336	GY	<i>diastaticus</i> var.			
<i>S. orientalis</i>		NRRL 2450	Y	<i>piger</i>		NRRL B-1775	GY
<i>S. ostreogriseus</i> ...		NRRL 2558	GY	<i>S. rutgersensis</i>		ATCC 3350	Y
<i>S. parvullus</i>		ATCC 12434	GY	<i>S. rutgersensis</i> ...			
<i>S. parvus</i>		NRRL B-1455	Y	subsp.			
<i>S. parvus</i>		ATCC 12433	Y	<i>castelarense</i> ...	X	ATCC 15191	GY
<i>S. parvus</i>		ATCC 12320	Y	<i>S. salmonicida</i>		NRRL B-1472	Y
<i>S. paucisporo-</i> ...				<i>S. saprophyticus</i> ...		ATCC 3351	Y
<i>genes</i>		ATCC 12596	Y	<i>S. saraceticus</i>		NRRL 2831	GY
<i>S. phaeochromo-</i> ...				<i>S. scabies</i>		ATCC 3352	GY
<i>genes</i>		ATCC 3338	R	<i>S. scabies</i>		ATCC 10246	Y
<i>S. phaeochromo-</i> ...				<i>S. setonii</i>		CBS	W
<i>genes</i>		NRRL B-1266	R	<i>S. sindenensis</i>		NRRL B-1866	Y
<i>S. phaeofaciens</i> ...	X	ATCC 15034	GY	<i>S. sioyaensis</i>		ATCC 13989	GY
<i>S. platensis</i>		ATCC 13865	GY	<i>S. spectabilis</i>		NRRL 2494	R
<i>S. pluri-</i>				<i>S. spheroides</i>		NRRL 2449	Y
<i>colorescens</i> ...		Hata	Y	<i>S. sulphureus</i>		NRRL B-1627	Y
<i>S. polychromo-</i> ...				<i>S. sulphureus</i>		ATCC 3007	Y
<i>genus</i>	X	ATCC 12595	B	<i>S. tanashiensis</i> ...		ATCC 15238	GY
<i>S. praecox</i>		ATCC 3374	Y	<i>S. umbrinus</i>		Gause 1703/53	R
<i>S. purpureo-</i> ...				<i>S. umbrosus</i>		NRRL 2791	GY
<i>chromogenus</i> ...		ATCC 3313	Y	<i>S. varsoviensis</i> ...		ATCC 14631	Y
<i>S. purpureo-</i> ...				<i>S. venezuelae</i>		ATCC 10595	R
<i>chromogenus</i> ...		CBS	Y	<i>S. venezuelae</i>		ATCC 10712	GY
<i>S. purpureo-</i> ...				<i>S. venezuelae</i>		ATCC 15439	GY
<i>chromogenus</i> ...		ATCC 3343	Y	<i>S. venezuelae</i>		ATCC 15068	R
<i>S. purpeofuscus</i> ...		NRRL B-1817	GY	<i>S. venezuelae</i>		NRRL 2718	R
<i>S. reticul.</i>		NRRL B-1253	Y	<i>S. versipellis</i>		NRRL 2528	GY
<i>S. reticuli</i>		IFO 3354	R	<i>S. verticillatus</i> ...		ATCC 13017	Y
<i>S. rimosus</i>		ATCC 10970	W	<i>S. vinaceus-</i> ...			
<i>S. rimosus</i>		ATCC 13224	W	<i>drappus</i>		NRRL 2363	GY
<i>S. rimosus</i> f.				<i>S. violaceoniger</i> ...		NRRL B-1476	GY
<i>paromomycinus</i> ..		ATCC 14827	W	<i>S. violaceoniger</i> ...		NRRL B-1477	GY
<i>S. rishiriensis</i> ...		ATCC 14812	GY	<i>S. violaceoniger</i> ...		NRRL B-1478	GY
<i>S. rochei</i>		NRRL B-1559	R	<i>S. violaceoniger</i> ...		NRRL 2834	GY

TABLE 1—Concluded

<i>Streptomyces</i> culture	Phalangi- form spores present	Source* and/or no.	Spore- color series†	<i>Streptomyces</i> culture	Phalangi- form spores present	Source* and/or no.	Spore- color series†
<i>S. violaceoruber</i> ...		ATCC 10147	GY	<i>S. willmorei</i>		ATCC 3332	Y
<i>S. violaceoruber</i> ...		ATCC 3355	GY	<i>Streptomyces</i> sp...		NRRL B-1289	GY
<i>S. violaceoruber</i> ...		NRRL B-122	GY	<i>Streptomyces</i> sp...		ATCC 11862	Y
<i>S. violaceoruber</i> ...		NRRL B-1257	GY	<i>Streptomyces</i> sp...		ATCC 11071	GY
<i>S. violaceus</i> var.				<i>Streptomyces</i> sp...		ATCC 11071a	GY
<i>aspartocinicus</i> ...		ATCC 13734	GY	<i>Streptomyces</i> sp...		IMRU 3676	R
<i>S. virginiae</i>	X	NRRL B-1446	R	<i>Streptomyces</i> sp...		NCIB 8766	GY
<i>S. virginiae</i>	X	IFO 3392	R	<i>Streptomyces</i> sp...		NRRL 2824	GY
<i>S. virginiae</i>	X	ATCC 12630	R	<i>Streptomyces</i> sp...		NRRL 2963	GY
<i>S. virginiae</i>	X	ATCC 13161	R	<i>Streptomyces</i> sp...	X	NCIB 9199	GY
<i>S. viridoflavus</i>		ATCC 12631	Y	<i>Streptomyces</i> sp...		ATCC 12463	GY
<i>S. willmorei</i>		ATCC 6867	Y	<i>Streptomyces</i> sp...		ATCC 15440	R

* ATCC, American Type Culture Collection; NRRL, Northern Regional Research Laboratory; IMRU, Institute of Microbiology, Rutgers University; CBS, Centraalbureau voor Schimmelf-cultures; Hata, Toju Hata, Kitasto Institute for Infectious Diseases, Tokyo; Gause, G. F. Gause, Institute of New Antibiotics, Moscow; IFO, Institute for Fermentation, Osaka; NCIB, National Collection of Industrial Bacteria.

† Abbreviations for spore-color series (5). GY, gray; R, red; Y, yellow; B, blue; W, white.

electron-dense areas seen under electron microscopy.

Serial thin-sectioning of the spores of *S. aureofaciens* has provided additional insight into the structure of this spore type. In longitudinal sections, the spores appear as relatively thick-walled cylinders that have been constricted at the center. Figure 2 shows a longitudinal thin section of a spore chain of *S. aureofaciens*, in which the abutting dumbbell-like, electron-dense elements are further illustrated. The fine structure, therefore, satisfactorily correlates with the phalangi-form shape seen in spores *in toto* and supports the validity of the form as a taxonomic implement.

In our studies of the spore morphology of smooth-spored streptomycetes, we have examined some 324 reference strains (comprising 205 named species and varieties) from various culture repositories. In addition, numerous soil isolates of several of these species have also been employed. Indicated in Table 1 are the organisms studied and the representatives of the 27 smooth-spored species (about 13%) that have shown the phalangi-form character. Differing with the species, the cylindrical spores of the organisms may range from more or less isodiametric elements to elongated cylindrical shapes. Although the phalangeal feature is most commonly associated with streptomycetes of the "Gray-spored" series (5), it may be noted that it has also been observed in other spore-color groups as well. In a few species having warty spores, typified by *S. lateritius*, this character was also encountered.

In a study of the spores of 48 strains of *S. aureofaciens*, it was learned that the phalangi-form morphology was present in all typical, wild-type strains. Occasionally, certain strains showed a fairly high proportion of nonphalangeal spores, e.g., simple elliptical to common elongated shapes; however, the phalangi-form types were generally present in sufficiently high ratios as to characterize the culture readily. This same situation also prevailed with other species in which multiple strains were examined. In a few species having phalangi-form spores, all reference strains bearing a given specific epithet did not exhibit the feature. However, upon consideration of other criteria, such nonconforming strains proved to be obviously of different specific taxa.

The phalangi-form spore type, though admittedly found in only a relatively small segment of the streptomycetes, appears distinctive and consistent enough to provide a useful taxonomic aid for differentiation of the species possessing it.

ACKNOWLEDGMENTS

We wish to express our appreciation to Jean Hayes for technical assistance, and to E. J. Backus for reviewing the manuscript.

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