Nutritional Characterization of Some Selected Actinomycetes

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Strains of several genetically important *Streptomyces* species and four strains of *Nocardia mediterranei* were screened for nutritional properties by using 150 different organic compounds. The methodology for an extensive nutritional analysis of actinomycetes is described, and the results are discussed on the basis of their taxonomic and ecological implications.

Actinomycetes constitute a substantial proportion of the microbial flora of soils. Among the strains that can be very easily isolated by conventional bacteriological techniques, the members of the economically important genus *Streptomyces* are particularly abundant and diverse and, consequently, a large number of species have been described. In the 8th edition of *Bergey's Manual of Determinative Bacteriology* 463 are described (9), and many more names could be added to the list. The assignment of a new strain to a given species described in the literature is often an exceedingly difficult task, and this unsatisfactory state of affairs perhaps can be mainly attributed to the formidable difficulty of defining the precise limits of species in this group of procaryotic organisms.

The numbers of actinomycetes in soil increase substantially after addition of organic matter. This, added to the enormous diversity of morphological and physiological attributes already known in the group, prompted us to explore the possibility of using an extensive nutritional analysis as a means of facilitating the phenotypic characterization of strains, thus providing a basis for their classification. A similar type of analysis proved of substantial help in the classification of other groups of nutritionally versatile organisms, such as the pseudomonads (13). The present communication describes the methodology used and an extensive nutritional characterization of several *Streptomyces* species and strains of *Nocardia mediterranei*. Since these organisms have been subjected to genetical analyses in other laboratories, results of our nutritional studies may prove useful for mapping purposes.

MATERIALS AND METHODS

Microbial strains. To facilitate the presentation of the results, the 25 strains used in our work have been designated with letters from *a* to *y*. Strains *a*, *g*, *h*, *i*, *j*, *k*, *n*, *o*, *p*, *q*, *r*, *s*, *t*, *u*, and *v* were received from D. A. Hopwood; the others were purchased from the American Type Culture Collection. Further details are given in Table 1.

Preparation of inocula for the nutritional screening. The cultures were grown on agar plates of a variety of culture media, including the medium of Hickey and Tresner (4), tomato medium, *Thermoactinomyces* fermentation medium, Difco ISP no. 2, and yeast-malt medium [tomato medium: tomato paste, 2%; glucose, 1%; K_2HPO_4 , 0.1%; $CaCO_3$, 0.2%; agar, 1.5%; pH 6.8 to 7.3. *Thermoactinomyces* fermentation medium: tryptone (Difco), 5 g; yeast extract, 2 g; Soytone (Difco), 2 g; white potato dextrin (soluble starch), 10 g; D-mannitol, 5 g; $MgSO_4$, 0.2 g; ferrous ammonium sulfate, 10 mg; $ZnCl_2$, 2.1 mg; $MnCl_2$, 1.8 mg; $CuSO_4$, 0.2 mg; $Co(NO_3)_2$, 0.5 mg; H_3BO_3 , 0.6 mg; distilled water, 1 liter; pH 7.2. Yeast-malt medium: yeast extract, 0.3%; malt extract, 0.3%; peptone, 0.5%; glucose, 1%; agar 2%; pH 6.7 to 7.8.] Spores, or vegetative growth in cases of poor sporulation, were harvested with an inoculating loop, and suspended in sterile distilled water. The suspensions were washed, and one drop of each suspension of washed cells was used as inoculum of the agar media, each of which contained a single carbon source.

Nutritional screening. The basal mineral medium described by Palleroni and Doudoroff (8) was used in all cases. This medium contains 0.033 M phosphate buffer (Na-K) (pH 6.8), 0.1% NH_4Cl , 0.05% $MgSO_4 \cdot 7H_2O$, 0.005% ferric ammonium citrate, and 0.0005% $CaCl_2$. For strain *h*, this medium was supplemented with cystine, methionine, and threonine (50 μ g of each per ml). The medium was solidified with 2% purified agar (Difco). A total of 150 organic compounds were tested for their capacity to serve as carbon and energy sources for growth. Each carbon source was added aseptically from a filter-sterilized concentrated solution to a portion of sterile basal mineral agar, to a final concentration of 0.1%. Exceptions were geraniol and naphthalene, which were not incorporated into the agar, but placed instead in the lid of the petri dish.

Various strains of actinomycetes can attack agar to a certain extent, and give appreciable growth in media without an added carbon source. The purified brand of agar gave a minimum background growth, but, even so, one of our strains (*S. coelicolor* strain *a*) grew noticeably more than the others in the unsupplemented medium. For this strain, the data obtained on the agar medium were rechecked by growth in liquid medium. The medium was dispensed in fractions of 10 ml in 50-ml Erlenmeyer flasks.

The inoculated media were incubated at 28°C. The flasks with liquid media were shaken with vigorous

TABLE 1. *Actinomycete strains used in the nutritional screening*

Species	Notation used in the tables	Hoffmann-La Roche collection no.	Original designation
<i>Streptomyces coelicolor</i>	<i>a</i>	2520A	Hopwood 1147
<i>S. coelicolor</i>	<i>b</i>	2525A	ATCC 3355
<i>S. coelicolor</i>	<i>c</i>	2526A	ATCC 10147
<i>S. coelicolor</i>	<i>d</i>	2527A	ATCC 19832
<i>S. coelicolor</i>	<i>e</i>	2534A	ATCC 23899
<i>S. coelicolor</i> subsp. <i>flavus</i>	<i>f</i>	2528A	ATCC 19894
<i>S. coelicolor</i>	<i>g</i>	2733A	Hopwood 1157
<i>S. coelicolor</i>	<i>h</i>	2741A	Hopwood J650 ^a
<i>S. violaceoruber</i>	<i>i</i>	2732A	Hopwood 1151
<i>S. parvulus</i>	<i>j</i>	2734A	Hopwood 2266
<i>S. glaucescens</i>	<i>k</i>	2522A	Hopwood 1472
<i>S. glaucescens</i>	<i>l</i>	2529A	ATCC 23622
<i>S. glaucescens</i>	<i>m</i>	2530A	ATCC 19905
<i>S. lividans</i>	<i>n</i>	2523A	Hopwood 1326
<i>S. rimosus</i>	<i>o</i>	2524A	Hopwood 1459
<i>S. rimosus</i>	<i>p</i>	2735A	Hopwood 2248
<i>S. rimosus</i>	<i>q</i>	2736A	Hopwood 2249
<i>S. rimosus</i>	<i>r</i>	2737A	Hopwood 2250
<i>S. rimosus</i>	<i>s</i>	2738A	Hopwood 2251
<i>S. rimosus</i>	<i>t</i>	2839A	Hopwood 2252
<i>S. rimosus</i>	<i>u</i>	2740A	Hopwood 2253
<i>Nocardia mediterranei</i>	<i>v</i>	2521A	Hopwood 1276
<i>N. mediterranei</i>	<i>w</i>	2531A	ATCC 21411
<i>N. mediterranei</i>	<i>x</i>	2532A	ATCC 21271
<i>N. mediterranei</i>	<i>y</i>	2533A	ATCC 27643

^a Mutant *agaA cysD mthB* obtained from strain *a*.

rotary motion (270 rpm). Growth was estimated visually on the solid and in liquid media after 7 and 21 days of incubation, and it was recorded as follows: —, no growth; +, abundant growth; and ±, scanty growth. All growth experiments were repeated at least once more for those compounds giving questionable growth.

All strains were tested for autotrophic growth in mineral medium in contact with an atmosphere of 52% hydrogen, 8% oxygen, 5% carbon dioxide, and 35% nitrogen gases.

RESULTS

The compounds utilized by all strains for growth were the following: glucose, mannose, fructose, maltose, lactose, starch, gluconate, succinate, fumarate, L-malate, citrate, α -ketoglutarate, pyruvate, glycerol, D-arabitol, mannitol, β -alanine, L-serine, L-threonine, L-glutamate, L-arginine, and L-ornithine.

Compounds not used by the strains as sole sources of carbon were: carbon dioxide, D-fucose, melezitose, inulin, cellulose, mucate, formate, isobutyrate, caproate, heptanoate, caprylate, pelargonate, caprate, oxalate, DL-tartrate, D-malate, itaconate, DL-tartrate, L-tartrate, glycollate, citraconate, itaconate, dulcitol, methanol, ethanol, *n*-propanol, isobutanol, secondary butanol, tertiary butanol, geraniol, D-mandelate, L-mandelate, benzoate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, phthalate, naphthalene, phenol, testoster-

one, α -aminobutyrate, α -aminovalerate, δ -aminovalerate, D-tryptophan, anthranilate, *p*-aminobenzoate, methylamine, butylamine, secondary butylamine, tertiary butylamine, isobutylamine, α -amylamine, benzylamine, tryptamine, creatine, nicotinate, isonicotinate, and trigonelline.

Compounds which inhibited background growth of *S. coelicolor* strain *a* in basal mineral agar were the following: formate, isobutyrate, caproate, heptanoate, caprylate, pelargonate, caprate, oxalate, DL-tartrate, D-malate, itaconate, benzoate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, phenylacetate, phenol, α -aminobutyrate, α -aminovalerate, D-tryptophan, anthranilate, *p*-aminobenzoate, tryptamine, and isonicotinate.

The compounds utilized by some of the strains are listed in Table 2. In this table the strains of the first, second, and fifth columns have been grouped according to their original designation as given in Table 1 (*S. coelicolor*, *S. violaceoruber*, and *S. lividans*, respectively). The nomenclature of these strains and their correct species assignment will be analyzed in the Discussion.

DISCUSSION

Waksman (14) refers to his own paper of 1919 as the first report of an attempt to investigate

TABLE 2. *Compounds utilized by a fraction of the strains*^a

Substrate	Strains <i>a</i> to <i>h</i> ^b (8 strains)	Strain <i>i</i> ^b (1 strain)	<i>S. parvu-</i> <i>lus</i> (1 strain)	<i>S. glau-</i> <i>cescens</i> (3 strains)	Strain <i>n</i> ^b (1 strain)	<i>S. rimosus</i> (7 strains)	<i>N. mediter-</i> <i>ranei</i> (4 strains)
Ribose	8	1	1	3	1	6 (<i>s</i>)	3 (<i>v</i>)
Xylose	8	1	1	3	1	5 (<i>o, r</i>)	4
D-Arabinose	0	0	0	0	0	2 (<i>o, p, s, t, u</i>)	4
L-Arabinose	8	1	1	3	1	5 (<i>p, r</i>)	4
L-Fucose	0	0	0	0	0	1 (<i>o, p, q, s, t, u</i>)	4
L-Rhamnose	7 (<i>e</i>)	1	1	3	1	1 (<i>o, p, r, s, t, u</i>)	4
Galactose	8	1	1	2 (<i>m</i>)	1	7	4
Sorbose	0	0	0	0	0	1 (<i>o, p, r, s, t, u</i>)	0
Sucrose	0	0	0	0	0	1 (<i>o, p, r, s, t, u</i>)	4
Cellobiose	8	1	1	3	1	6 (<i>r</i>)	4
Melibiose	6 (<i>a, e</i>)	1	1	1 (<i>k, m</i>)	0	6 (<i>o</i>)	1 (<i>v, w, x</i>)
Salicin	8	1	1	3	1	4 (<i>o, p, s</i>)	4
Raffinose	2 (<i>a, c, d, e, f, g</i>)	1	0	0	0	6 (<i>o</i>)	1 (<i>w, x, y</i>)
2-Ketogluconate	1 (<i>a, b, c, d, e, g, h</i>)	0	0	0	0	0	1 (<i>v, w, x</i>)
Saccharate	3 (<i>a, e, f, g, h</i>)	1	1	0	1	0	1 (<i>v, w, x</i>)
Lactobionate	6 (<i>e, g</i>)	1	1	3	1	2 (<i>o, p, r, s, t</i>)	4
Glucosamine	7 (<i>f</i>)	1	1	3	1	7	4
Acetate	8	1	1	3	1	7	2 (<i>w, x</i>)
Propionate	8	1	1	2 (<i>k</i>)	1	6 (<i>o</i>)	3 (<i>x</i>)
Butyrate	2 (<i>a, b, c, e, g, h</i>)	0	0	0	0	0	0
Valerate	2 (<i>a, b, c, d, g, h</i>)	0	0	0	0	0	0
Isovalerate	4 (<i>a, b, c, h</i>)	1	1	0	0	4 (<i>o, p, r</i>)	0
Malonate	8	1	1	0	1	0	0
Maleate	0	0	0	0	0	3 (<i>o, p, q, t</i>)	0
Adipate	6 (<i>a, h</i>)	0	0	0	1	0	2 (<i>w, y</i>)
Pimelate	2 (<i>a, b, d, e, g, h</i>)	0	0	2 (<i>k</i>)	0	0	4
Suberate	8	1	1	1 (<i>k, m</i>)	1	1 (<i>o, p, r, s, t, u</i>)	3 (<i>w</i>)
Sebacate	8	1	1	1 (<i>k, m</i>)	1	2 (<i>o, p, r, s, t</i>)	3 (<i>x</i>)
D-Malate	3 (<i>a, d, e, g, h</i>)	1	1	2 (<i>l</i>)	0	5 (<i>o, p</i>)	4
<i>m</i> -Tartrate	0	0	0	0	0	6 (<i>p</i>)	0
β -OH-butyrate	8	1	1	3	1	6 (<i>p</i>)	4
Lactate	6 (<i>d, f</i>)	1	0	1 (<i>l, m</i>)	1	5 (<i>p, r</i>)	4
Glycerate	2 (<i>a, b, c, d, f, h</i>)	0	0	0	0	0	2 (<i>v, x</i>)
Hydroxymethyl glutarate	1 (<i>a, b, c, d, e, f, g</i>)	0	0	0	0	1 (<i>o, p, q, r, s, t</i>)	0
Aconitate	6 (<i>b, d</i>)	1	1	3	1	7	4
Levulinate	8	1	1	3	1	5 (<i>o, r</i>)	3 (<i>x</i>)
Propylene glycol	5 (<i>e, f, g</i>)	1	0	0	1	0	0
Erythritol	0	0	0	0	0	7	0
Xylitol	0	0	0	0	0	6 (<i>r</i>)	0
Adonitol	0	0	0	0	0	6 (<i>r</i>)	0
Sorbitol	3 (<i>b, c, d, e, f</i>)	1	0	0	0	7	0
<i>m</i> -Inositol	7 (<i>e</i>)	1	1	1 (<i>k, m</i>)	1	7	4
Isopropanol	1 (<i>a, b, c, d, e, g, h</i>)	0	0	0	0	0	0
<i>n</i> -Butanol	0	0	0	2 (<i>k</i>)	0	0	2 (<i>v, x</i>)
<i>p</i> -OH-benzoate	6 (<i>e, g</i>)	1	1	1 (<i>k, m</i>)	1	4 (<i>o, p, r</i>)	1 (<i>w, x, y</i>)
Phenylacetate	4 (<i>a, b, c, h</i>)	0	0	0	0	0	0
Quinate	0	0	1	0	0	1 (<i>o, p, r, s, t, u</i>)	4
Glycine	7 (<i>g</i>)	1	1	3	1	7	4
L-Alanine	8	1	1	3	1	6 (<i>q</i>)	4
D-Alanine	4 (<i>c, d, e, g</i>)	0	0	1 (<i>l, m</i>)	1	6 (<i>s</i>)	4
L-Leucine	6 (<i>d, f</i>)	1	1	1 (<i>l, m</i>)	1	3 (<i>o, p, s, t</i>)	4
L-Isoleucine	7 (<i>g</i>)	1	1	2 (<i>k</i>)	0	5 (<i>o, p</i>)	4

TABLE 2—Continued

Substrate	Strains <i>a</i> to <i>h</i> ^b (8 strains)	Strain <i>i</i> ^b (1 strain)	<i>S. parvu-</i> <i>lus</i> (1 strain)	<i>S. glau-</i> <i>cescens</i> (3 strains)	Strain <i>n</i> ^b (1 strain)	<i>S. rimosus</i> (7 strains)	<i>N. mediter-</i> <i>ranei</i> (4 strains)
L-Norleucine	0	0	0	0	0	0	3 (<i>x</i>)
L-Valine	6 (<i>a, h</i>)	1	1	3	1	7	4
L-Aspartate	8	1	1	3	1	6 (<i>q</i>)	4
D-Aspartate	4 (<i>b, d, f, g</i>)	1	0	0	1	0	2 (<i>v, y</i>)
D-Glutamate	6 (<i>e, g</i>)	0	0	0	1	1 (<i>p, q, r, s,</i> <i>t, u</i>)	4
L-Asparagine	7 (<i>h</i>)	1	1	3	1	7	4
L-Lysine	8	1	1	2 (<i>m</i>)	0	7	4
L-Citrulline	3 (<i>a, d, e, g, h</i>)	0	1	2 (<i>k</i>)	0	4 (<i>p, r, s</i>)	3 (<i>w</i>)
α -Aminovalerate	0	0	0	0	0	0	1 (<i>w, x, y</i>)
L-Tyrosine	5 (<i>a, d, h</i>)	1	1	2 (<i>m</i>)	1	7	2 (<i>v, w</i>)
L-Tryptophan	1 (<i>a, b, c, d,</i> <i>e, g, h</i>)	0	0	0	0	0	4
Kynurenate	3 (<i>a, b, c, d, h</i>)	0	1	0	0	0	0
Ethanolamine	8	1	1	2 (<i>k</i>)	0	7	4
Putrescine	8	1	1	2 (<i>m</i>)	1	6 (<i>r</i>)	1 (<i>v, x, y</i>)
Spermine	6 (<i>e, g</i>)	1	1	1 (<i>k, m</i>)	1	6	2 (<i>w, x</i>)
Histamine	2 (<i>b, c, d, e, f, g</i>)	0	0	0	0	0	0
Betaine	2 (<i>b, c, d, e, f, g</i>)	0	0	0	0	0	2 (<i>w, x</i>)
Sarcosine	3 (<i>b, c, e, f, g</i>)	0	0	0	0	1 (<i>p, q, r, s,</i> <i>t, u</i>)	4
Hippurate	3 (<i>a, d, e, g, h</i>)	0	0	0	0	1 (<i>o, p, r, s, t,</i> <i>u</i>)	2 (<i>w, x</i>)
Pantothenate	8	1	1	3	1	7	3 (<i>v</i>)
Acetamide	0	0	0	0	0	1 (<i>o, p, q, s,</i> <i>t, u</i>)	0

^a Numbers of positive strains for each substrate. Negative strains given in parentheses.^b See Table 3 for correct identification of strains.

the ability of different species of actinomycetes to utilize carbon compounds for growth. The early method was later changed by various workers mainly with respect to the composition of the basal medium and the precautions recommended to insure a uniform distribution of the inoculum. At present, two different criteria are followed by actinomycete taxonomists for the investigation of the utilization of various low-molecular-weight organic compounds. Many microbiologists follow the methods used by collaborators of the International Streptomyces Project (12). These methods include the so-called carbon utilization tests in which growth on 10 carbohydrates is determined. Other workers prefer to follow instead the methodology recommended by Gordon (3) in which the change in color of an indicator added to the medium rather than growth is recorded. The two methodologies are not mutually exclusive, and it is therefore surprising that some eclectic temperament has not emerged so far, particularly when the taxonomy of the group is one of the most difficult in the microbial world and microbiologists should, in principle, welcome any well founded source of phenotypic information.

The list of compounds proposed by Gordon includes some organic acids and carbohydrates. The utilization of an organic anion makes the

medium more alkaline. A survey on the utilization of six organic acids, based in part on the Gordon methodology, was performed on 118 organisms by Robbel and Kutzner (11). Transformations of sugars generally give an acid reaction to the medium. Both reactions have been used extensively in the identification and classification of other microbial groups, and they are very clear, sensitive, easy to read, and generally very reproducible. The two reactions differ, however, in the type of information that they give. Whereas an alkaline reaction in the medium gives a sensitive indication of the utilization of the organic anion, acid production from sugars is not necessarily correlated with the utilization of the carbon compound. For instance, an organism may be able to oxidize a sugar to a sugar acid without extracting any benefit from the conversion, or it may degrade rather extensively the carbohydrate, producing one or several acids with better chances of utilizing the intermediates for growth. It is true that the practical taxonomist does not need to worry about these complications, since in most cases there is no need of a thorough understanding of the mechanisms underlying the phenotypic traits, as long as the detection and recording of the results are free of ambiguities. There is, however, a serious objection to the value of the test in the possibility of

oxidation of various aldose sugars to the respective aldonic acids by the same enzymatic system. When used blindly for any collection of aldose sugars, the test can therefore yield redundant results in some cases.

One of the few attempts at extensive nutritional characterization of strains of actinomycetes has been the work of Goodfellow (2), who studied the capacity of a large number (102) of carbon compounds to serve as growth substrates of a collection of nocardioform organisms. The methodology presented here for the extensive analysis of nutritional properties of actinomycetes has been found to be quite reliable. Since roughly one-third of the compounds assayed are acids, the method could be further improved by incorporation of an appropriate acid-base indicator to the media containing anionic substrates.

A number of strains do not replicate satisfactorily with the velvet technique of Lederberg and Lederberg (7), and we have not attempted to use it in the present case, since the number of strains under study was small. For larger numbers, it may be possible to speed up considerably the inoculation of the plates by the method of replica plating, using well sporulated master cultures.

Some *Streptomyces* strains give appreciable growth in agar of mineral media without added carbon sources. Additional growth over this background is often very difficult to judge, although a trained eye can discern growth due to the added carbon source in the great majority of cases. In the group of strains examined by us, *S. coelicolor* strain *a* presented this problem in its most acute form. Hopwood (5) mentions that this strain [originally designated A3(2)] is derived from a group of agar-decomposing organisms studied by Stanier in 1942. For this strain the whole screening was repeated in liquid medium, and the results were essentially the same as those obtained with the solid medium. The only significant discrepancies were in the utilization of D-alanine, D-aspartate, and citrate, all of which gave better growth in solid than in liquid medium. Strain *h* of this collection is derived from strain *a*, and it is characterized by the inability to decompose agar. Consequently, it only gives a faint marginal growth in our control plates. As indicated in Materials and Methods, the mineral medium for this particular strain has been supplemented with cystine, methionine, and threonine, which satisfy its auxotrophic requirements. This strain has essentially the same nutritional spectrum as its parent strain *a*, although there are a few differences which are apparently unrelated to the properties recorded in its known genotype.

TABLE 3. General phenotypic properties of strains labeled *S. coelicolor*, *S. violaceoruber*, and *S. lividans* (strains *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, and *n*)

Original designation	Color of spore mass	Shape of spore chains ^a	Diffusible blue pigment(s) in Hickey and Tresner (4) medium	Utilization of			Correct designation
				L-Rhamnose	Raffinose	m-Inositol	
<i>S. coelicolor</i> , strain <i>a</i>	Gray	S	+	+	-	+	<i>S. violaceoruber</i>
<i>S. coelicolor</i> , strain <i>b</i>	Gray	RF to S	+	+	+	+	<i>S. violaceoruber</i>
<i>S. coelicolor</i> , strain <i>c</i>	Gray	RA to S	-	+	-	+	<i>S. violaceoruber</i>
<i>S. coelicolor</i> , strain <i>d</i>	Gray	S	+	+	-	+	<i>S. violaceoruber</i>
<i>S. coelicolor</i> , strain <i>e</i>	Yellow	RF	-	-	-	+	<i>S. coelicolor</i>
<i>S. coelicolor</i> subsp. <i>flavus</i> , strain <i>f</i>	Yellow	RF	-	+	-	+	<i>S. coelicolor</i>
<i>S. coelicolor</i> , strain <i>g</i>	Yellow	RF	-	+	-	+	<i>S. coelicolor</i>
<i>S. coelicolor</i> , strain <i>h</i>	Gray	S	+	+	+	+	<i>S. violaceoruber</i>
<i>S. violaceoruber</i> , strain <i>i</i>	Gray	S	+	+	+	+	<i>S. violaceoruber</i>
<i>S. lividans</i> , strain <i>n</i>	Gray	S (Loose spirals and hooks)	-	+	-	+	<i>S. violaceoruber</i>

^a Abbreviations: S, spirals; RF, *rectus flexibilis* (straight, flexible); RA, *retinaculum apertum* (open loops).

TABLE 4. *Substrates of probable taxonomic importance for the differentiation of at least two of the species^a*

Substrate	<i>S. coelicolor</i> (3 strains)	<i>S. violaceoruber</i> (7 strains)	<i>S. parvulus</i> (1 strain)	<i>S. glaucescens</i> (3 strains)	<i>S. rimosus</i> (7 strains)	<i>N. mediterranei</i> (4 strains)
D-Arabinose	—	—	—	—	2/7	+
L-Fucose	—	—	—	—	1/7	+
Sucrose	—	—	—	—	1/7	+
Saccharate	—	5/7	+	—	—	1/4
Malonate	+	+	+	—	—	—
Adipate	+	4/7	—	—	—	2/4
Pinelate	1/3	1/7	—	2/3	—	+
Lactate	2/3	6/7	—	1/3	5/7	+
Propylene glycol	—	+	—	—	—	—
Erythritol	—	—	—	—	+	—
Xylitol	—	—	—	—	6/7	—
Adonitol	—	—	—	—	6/7	—
Sorbitol	1/3	3/7	—	—	+	—
Quinate	—	—	+	—	1/7	+
D-Alanine	1/3	4/7	—	1/3	6/7	+
D-Glutamate	1/3	6/7	—	—	1/7	+
L-Tryptophan	1/3	—	—	—	—	+
Sarcosine	—	3/7	—	—	1/7	+

^a Symbols: +, all strains grow; —, no strain grows. When the property is not universal, the fraction of positive strains is given.

The strains examined by us are only moderately versatile, the total number of compounds used for growth ranging from about 50 to nearly 80. The groups of compounds that are most frequently used are the carbohydrates and the amino acids. The utilization of other classes of substrates is rather irregular and, characteristically, many fatty acids, alcohols, aromatic compounds, and miscellaneous nitrogenous compounds did not support growth of any of the strains examined. Some substrates (particularly some aliphatic acids and aromatic compounds) may actually be toxic at the concentration used in the media, as suggested by the inhibition of background growth of strain *a* when one compares growth on these compounds with that obtained on the unsupplemented mineral agar.

Unpublished results obtained by us on a larger collection of strains of *Streptomyces* and of other genera of actinomycetes suggest that the nutritional versatility is in general very similar to that of the strains examined here. In comparison, some aerobic pseudomonads, notably some fluorescent organisms and also *Pseudomonas cepacia* and *P. pseudomallei*, appear to be more versatile (1, 10, 13). These organisms, which are endowed with a fast growth rate, would predominate in the soil microflora when low-molecular-weight organic compounds are being released as a consequence of the hydrolytic activities of other microorganisms, particularly fungi and actinomycetes.

Actinomycetes have a reputation for marked nutritional versatility which is in part supported by the results of our analysis, although it appears

rather surprising that some classes of organic compounds that are excellent substrates for other common organisms do not support growth of any of the strains in this collection. Extremely slow growth at the expense of some substrates may be recorded as a negative result in our experiments. It is possible that some substrates may be used when they are present in the medium at higher or lower concentrations than the one used here, or in mixtures with other cometabolizable carbon sources. But it also appears likely that actinomycetes may be able to grow on a number of unusual compounds not normally included in nutritional tests. A large variety of miscellaneous organic molecules resistant to the attack by most other groups of microorganisms, which may be present in natural habitats, and many synthetic compounds may be growth substrates for actinomycetes. Inclusion of these carbon sources in a nutritional screening may be the only way to reveal the nutritional potentiality of the group.

In Table 1 we have named the strains included in the present study according to the designation given to them at the source of origin. The nomenclatural state of several of these strains is at present rather inadequate, and some comments on this point will not be out of place here. The species *S. coelicolor* and *S. violaceoruber* can be differentiated by using the criteria discussed by Kutzner and Waksman (6). The most useful characters appear to be the morphology and color of the spore mass, the properties of the pigments produced, and the utilization of L-rhamnose, *m*-inositol, and raffinose as carbon

sources for growth. An analysis of some of these basic properties for various strains used in our work is given in Table 3, which also includes the information on *S. lividans*, a name considered to be a synonym of *S. violaceoruber*. According to data published in the literature, *S. violaceoruber* can be distinguished by the formation of chains of spores with the shape of spirals, by the gray color of the spore mass, and by the utilization of the three carbon sources L-rhamnose, *m*-inositol, and raffinose. Instead, *S. coelicolor* does not form spirals, produces a spore mass of yellowish color, and does not use any of the three carbon sources. As seen in Table 3, the diagnoses of strains *b*, *e*, *h*, and *i* is clear-cut. Strains *c*, *d*, and *n* form spirals and gray spore masses but are unable to use raffinose, although perhaps this trisaccharide may be used at higher concentrations than the one used in our experiments. Finally, strains *f* and *g* have the *S. coelicolor* morphology and pigmentation, but they appear atypical in the capacity of utilization of L-rhamnose and *m*-inositol.

If we accept the designation of these strains as presented in the last column of Table 3, some interesting correlations in nutritional properties are worth discussing. Propylene glycol is a substrate not used by any of the three strains of *S. coelicolor*, and it is used by all the *S. violaceoruber* strains. The *S. coelicolor* strains *e* and *g* differ from strain *f* and from all the *S. violaceoruber* strains in being able to use glucosamine but lacking the capacity of growth on lactobionate, *p*-hydroxybenzoate, and spermine. The behavior of strain *f*, which was received under the designation *S. coelicolor* subsp. *flavus*, is rather atypical. In addition to the above differences, it also differs from the other two *S. coelicolor* strains in the utilization of 2-ketogluconate, butyrate, pimelate, D-malate, lactate, glycerate, isopropanol, D-alanine, L-leucine, L-citrulline, L-tryptophan, and hippurate (Table 2). These substrates, however, do not have diagnostic importance for the differentiation of *S. coelicolor* and *S. violaceoruber*.

D-Arabinose, L-fucose, sucrose, and quinate are substrates used by the four strains of *Nocardia mediterranei* and by relatively few of the *Streptomyces* strains. Strains of *S. rimosus* are the only ones in the collection that can grow with *m*-tartrate, erythritol, xylitol, and adonitol. Outside of these exceptional situations, however, no nutritional pattern can be drawn for a clear-cut separation of the species. Characters of probable taxonomic significance for the differentia-

tion of at least two species are presented in Table 4. Nevertheless, we want to emphasize that our study has been restricted to the examination of relatively few strains that are familiar to geneticists, and therefore it would be pretentious to draw taxonomic conclusions from the results. Without a better knowledge of the intraspecific variability, our data are of limited value for a definition of the nutritional phenotypes of the species involved.

REPRINT REQUESTS

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