Probabilistic identification of streptomycetes using miniaturized physiological tests

PETER KÄMPFER¹* and REINER M. KROPPESTEDT²

¹ Fachgebiet Hygiene, Technische Universität Berlin, Amrummerstr. 32, D-1000 Berlin 65, Federal Republic of Germany

² Deutsche Sammlung von Mikroorganismen und Zellkulturen, Marscheroderweg 1b, D-3300 Braunschweig, Federal Republic of Germany

(Received 26 October 1990; accepted 13 February 1991)

A frequency matrix of positive results for phena defined in a previous phenetic classification was constructed. A total of 329 physiological characters from 782 strains was taken as the basis for this identification matrix. The minimum number of diagnostic characters for the matrix was selected using computer programs for calculation of different separation indices (CHARSEP) and selection of group diagnostic properties (DIACHAR). The resulting matrix consisted of 52 phena versus 50 characters. Overlap of phena was found to be relatively small (OVERMAT program). Identification scores for the most typical hypothetical organism of each phenon was satisfactory (MOSTTYP program). The matrix was evaluated theoretically and practically (MATIDEN program). For members of major clusters and subclusters, e.g. *Streptomyces albidoflavus*, *S. halstedii*, *S. griseus*, *S. fradiae*, *S. rimosus* and *S. albus*, identification scores were high. Organisms of phena containing only small numbers of strains could be identified correctly, but with lower accuracy. The identification rate of the matrix (Willcox probability > 0.90) in the theoretical evaluation was 84.39%, and in the practical evaluation 78.12%.

Introduction

Current classification and identification of species within the genera *Streptomyces* and *Streptoverticillium* is based on the numerical taxonomic studies provided by Williams *et al.* (1983a) and Locci *et al.* (1981). Because of the heterogeneity of strains grouped within one species, a correct identification is polythetic and can only be obtained by probabilistic methods. Three identification matrices (Williams *et al.*, 1983b; Langham *et al.*, 1989a) were constructed on the basis of the results of the numerical study of Williams *et al.* (1983a) for members of the genus *Streptomyces*. For identification of members of the genus *Streptoverticillium*, an additional probability matrix was provided (Williams *et al.*, 1985). Although these matrices were objectively constructed and technically sound, they depend on the numerical classifications of Locci *et al.* (1981) and Williams *et al.* (1983a), which included 111 *Streptoverticillium* and 394 *Streptomyces* strains, respectively. The probability matrix of Williams *et al.* (1983b) was constructed only for major phena, and

identification of members of the minor clusters required the application of an additional matrix (Langham *et al.*, 1989a).

The more comprehensive numerical survey of Kämpfer *et al.* (1991) (accompanying paper), including 821 strains of the genera *Streptomyces* and *Streptoverticillium*, confirmed many of the groups found by Williams *et al.* (1983a), but several of the phena containing only a few strains were not detected. The taxonomic position of many strains not included in the study of Williams *et al.* (1983a) and currently classified within species category IV (Williams *et al.*, 1989) was also evaluated. The results of the study by Kämpfer *et al.* (1991) further supported the view that strains placed in the genus *Streptoverticillium*, as described by Locci & Schofield (1989), should be reclassified into the genus *Streptomyces*, which still seems to be overspeciated.

As identification depends heavily on classification (Sneath, 1974; Goodfellow, 1986; Hill, 1974), the results of the numerical study of Kämpfer *et al.* (1991) were used to construct a single probability matrix for the numerical identification of streptomycetes using miniaturized physiological tests for members of all major and minor phena.

Abbreviations: OTU, operational taxonomic unit; p-NA, *p*-nitro-anilide; p-NP, *p*-nitrophenyl.

Table 1. A percentage probability matrix for *Streptomyces* species

No. of cluster or subcluster	Phenon	No. of strains	Utilization of: N-Acetyl-D-galactosamine	L-Arabinose	L-Fucose	D-Glucarate	D-Glucuronate	Lactose	Lactulose	L-Lyxose	α -L-Melibiose	Methyl α -D-glucopyranoside	Methyl α -D-mannoside	α -D-Raffinose	L-Rhamnose	D-Ribose	D-Sucrose	D-Turanose	Adonitol	D-Arabitol	D-Inositol	Maltitol
1-1	<i>S. albidoflavus</i>	(52)	75	99	8	2	2	96	85	1	2	42	1	2	1	94	54	69	10	99	27	46
1-2	<i>S. halstedii</i>	(6)	67	99	17	1	50	99	33	17	1	1	1	1	50	99	1	17	1	50	1	33
1-3	<i>S. griseus</i>	(81)	95	40	21	5	41	69	22	23	1	12	1	1	52	99	1	9	52	91	6	6
1-4	<i>S. olivaceus</i>	(6)	83	99	99	99	33	67	17	83	1	1	1	1	83	99	1	1	1	83	33	1
1-5	<i>S. antibioticus</i>	(19)	32	99	26	1	21	89	21	1	79	1	26	37	11	95	21	11	1	95	16	5
1-6	<i>S. flaveolus</i>	(16)	44	75	1	6	50	75	62	19	50	1	19	19	44	94	12	1	1	99	50	1
1-7	<i>S. diastatochromogenes</i>	(25)	32	99	20	12	68	96	92	44	64	4	4	48	80	96	36	12	12	80	64	1
2	<i>S. exfoliatus</i>	(30)	83	87	33	7	23	80	27	1	20	1	3	17	43	57	27	20	1	3	1	1
3	<i>S. gelaticus</i>	(3)	99	33	99	1	33	67	33	1	99	99	33	33	99	99	67	67	67	33	1	99
4	<i>S. graminofaciens</i>	(6)	67	99	50	17	83	99	67	33	99	83	67	99	83	99	99	83	17	83	67	83
5	<i>S. parvus</i>	(5)	99	20	40	20	1	1	1	20	1	1	20	20	20	99	1	1	20	99	20	1
6	<i>S. rochei</i>	(79)	65	99	29	35	78	97	94	70	54	62	47	18	95	95	44	63	4	99	92	51
7	<i>S. prasinopilosus</i>	(7)	29	99	43	1	43	99	71	86	14	86	14	1	86	99	71	14	1	99	99	14
8	<i>Streptomyces</i> sp.	(4)	50	99	99	25	1	75	75	75	99	1	1	99	99	99	99	1	1	75	75	1
9	<i>S. violaceus</i>	(118)	64	98	92	42	74	97	90	67	96	24	25	92	92	99	83	34	64	96	99	17
10	<i>S. sparsogenes</i>	(2)	99	99	99	99	99	99	99	1	50	1	1	99	99	99	99	1	99	99	99	1
11	<i>S. psammoticus</i>	(5)	80	99	80	1	40	99	99	99	80	20	1	80	99	99	80	40	20	99	60	20
12	<i>S. aurantiacus</i>	(2)	50	99	50	99	1	99	99	99	99	50	99	99	99	50	1	99	1	99	99	50
13	<i>S. niveoruber</i>	(9)	99	99	78	67	78	99	99	89	89	99	99	89	99	99	67	44	11	99	99	56
14	<i>Streptomyces</i> sp.	(2)	1	99	99	1	1	99	99	50	99	1	1	99	99	99	99	1	1	1	99	1
17	<i>S. griseoloalbus</i>	(2)	1	99	50	50	50	99	99	50	1	50	1	1	99	99	1	1	1	99	50	99
20	<i>S. chrysomallus</i>	(6)	33	67	33	17	50	67	17	17	50	33	1	50	33	99	50	1	50	99	17	
21	<i>S. thermovulgaris</i>	(5)	20	80	20	20	40	80	20	1	60	60	20	20	20	60	80	80	1	99	99	80
22-1	<i>Sv. griseocarneum</i>	(51)	43	1	1	1	2	4	1	1	1	1	22	1	1	96	4	1	4	2	94	1
22-2	<i>S. roseoviridis</i>	(5)	60	60	1	20	1	99	40	1	20	1	1	1	20	20	1	1	1	20	20	1
22-3	<i>S. lavendulae</i>	(43)	53	47	7	1	16	12	9	1	12	1	1	1	12	19	14	9	23	2	21	2
22-4	<i>S. misakiensis</i>	(17)	53	65	18	1	12	41	12	1	1	1	1	18	1	12	59	24	1	1	1	1
22-5	<i>S. fradiae</i>	(17)	6	76	1	1	6	35	12	1	1	1	1	1	6	35	6	12	1	1	6	1
23	<i>S. griseolus</i>	(2)	50	1	1	1	50	1	1	50	50	1	1	50	1	99	50	50	50	50	50	50
24	<i>S. fungicidicus</i>	(4)	50	25	99	25	1	50	75	25	99	1	1	99	25	75	25	1	50	50	99	1
25	<i>S. lydicus</i>	(17)	82	24	65	1	18	47	59	1	82	47	1	76	1	94	82	71	47	82	88	71
26	<i>S. flavidovirens</i>	(2)	99	50	50	1	1	1	1	1	1	1	1	1	1	99	99	50	99	1	99	1
27	<i>S. fradiae</i>	(2)	99	99	1	1	50	1	1	1	1	1	1	1	1	99	50	1	1	1	1	1
28	<i>S. prasinus</i>	(2)	50	99	1	1	99	99	99	50	99	1	99	99	99	99	1	1	1	99	1	1
30	<i>S. albus</i>	(21)	99	24	43	1	19	95	95	1	10	86	86	10	10	95	62	71	99	90	81	86
33	<i>S. albidoflavus</i>	(19)	95	99	79	11	79	47	26	11	84	68	32	84	21	99	21	68	79	95	79	63
35	<i>S. rimosus</i>	(26)	96	38	8	1	27	62	54	1	65	1	54	69	1	99	4	38	92	96	81	19
41	<i>S. endus</i>	(2)	50	99	1	50	50	99	1	1	99	99	99	99	50	99	50	99	50	99	50	99
44	<i>Streptomyces</i> sp.	(2)	99	99	50	1	99	50	99	99	99	1	1	50	99	99	1	1	50	99	99	1
49	<i>S. roseiscleroticus</i>	(2)	99	99	1	1	99	99	99	50	99	99	1	1	99	99	99	99	1	99	50	99
50	<i>S. violatus</i>	(2)	99	99	99	1	1	99	99	50	99	1	1	99	50	99	99	50	99	99	99	50
51	<i>S. violaceusniger</i>	(17)	88	99	94	94	88	99	71	76	94	94	47	88	88	99	41	29	59	88	82	65
64	<i>S. longissimus</i>	(3)	99	99	99	1	99	99	67	33	67	33	99	1	33	99	99	67	33	99	99	67
65	<i>Streptomyces</i> sp.	(2)	99	99	99	99	99	99	99	50	99	50	99	99	99	99	99	99	99	99	99	99
66	<i>S. flocculus</i>	(3)	1	99	1	99	1	33	67	33	67	1	33	67	67	99	99	99	1	67	1	33
68	<i>S. sulphureus</i>	(2)	1	50	1	1	1	99	1	1	50	50	1	50	50	99	50	50	99	50	50	50
69	<i>S. ochraceiscleroticus</i>	(10)	90	99	60	99	99	99	90	60	70	90	20	99	99	99	99	90	99	99	99	99
76	<i>Streptomyces</i> sp./ <i>Nocardia</i> sp.	(9)	1	1	1	11	1	1	1	1	1	1	1	1	11	67	1	1	1	1	1	1
78	<i>S. fragilis</i>	(2)	1	99	50	1	50	1	1	1	50	1	1	1	1	50	50	1	1	1	1	1
79	<i>S. lipmanii</i>	(3)	99	33	67	1	67	1	1	1	1	1	1	1	1	33	1	1	1	1	33	1
80	<i>Streptomyces</i> sp.	(3)	67	67	33	1	1	33	1	1	33	33	1	1	67	1	33	1	33	33	33	1
82	<i>Streptomyces</i> sp.	(2)	1	1	1	1	1	1	1	1	1	1	1	1	50	1	1	1	1	50	1	1

|--|

Table 2. A diagnostic table for *Streptomyces* species defined

Single-member cluster	Utilization of: N-Acetyl-D-galactosamine	L-Arabinose	L-Fucose	D-Glucarate	D-Glucuronate	Lactose	Lactulose	L-Lyxose	α -L-Melibiose	Methyl α -D-glucopyranoside	Methyl α -D-mannoside	α -D-Raffinose	L-Rhamnose	D-Ribose	D-Sucrose	D-Turanose	Adonitol	D-Arabitol	<i>i</i> -Inositol	Maltitol
<i>Sv. luteoreticuli</i> ISP 5509	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	-
<i>S. massaporeus</i> ISP 5035	-	+	-	-	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-
<i>S. lanatus</i> ISP 5090	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-
<i>S. griseoruber</i> ISP 5281	-	+	+	-	-	+	+	+	+	-	-	-	+	+	-	-	-	-	+	-
<i>S. cyaneogriseus</i> ISP 5534	+	+	+	+	-	-	-	+	+	-	-	-	+	+	+	-	-	-	-	-
<i>S. loidensis</i> DSM 40825	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	+	+	-
<i>S. albus</i> ISP 5313 ^T	+	-	-	-	-	+	+	-	-	-	-	-	-	+	+	+	+	+	+	-
<i>S. fulvissimus</i> ISP 5593 ^T	+	+	+	+	+	+	-	+	+	+	-	+	-	+	+	+	+	+	+	+
<i>S. varsoviensis</i> ISP 5346 ^T	-	-	+	-	+	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-
<i>S. luteofluorescens</i> ISP 5398	+	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-
<i>S. caelicus</i> DSM 40835	+	+	+	+	-	+	-	+	+	-	-	-	+	+	-	-	-	+	+	-
<i>S. sphaeroides</i> ISP 5292	+	-	+	+	-	-	-	+	+	-	+	-	+	+	+	+	+	+	-	+
<i>S. olivaceus</i> ISP 5072	-	+	-	-	+	+	-	-	-	-	+	-	+	+	+	+	-	+	+	+
<i>S. niveus</i> ISP 5088	+	+	+	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	+	+
<i>S. scabies</i> DSM 40961	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-
<i>S. pallidus</i> ISP 5531	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-	+	+	+	-
<i>S. anulatus</i> ISP 5361 ^T	+	+	+	+	-	+	+	-	+	-	-	+	+	+	+	-	-	+	-	-
<i>S. parvulus</i> DSM 40722	-	+	-	-	+	+	+	-	-	-	+	-	+	+	-	-	-	+	+	-
<i>S. bluensis</i> ISP 5564	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	-	-	+	+	+
<i>S. hygroscopicus</i> DSM 40822	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
<i>S. caeruleus</i> ISP 5103 ^T	-	+	-	-	-	-	+	-	+	+	-	-	+	+	+	-	-	+	-	-
<i>Streptomyces</i> sp. DSM 40966	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-
<i>S. malachiticus</i> ISP 5167	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	+	-
<i>S. bellus</i> ISP 5185	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
<i>S. xantholiticus</i> ISP 5244	+	-	-	-	-	+	+	+	+	-	-	+	-	-	-	-	-	-	+	-
<i>S. albosporeus</i> DSM 40975 ^T	+	+	+	+	-	+	-	+	+	-	+	-	+	-	-	+	-	+	+	+
<i>Nocardia</i> sp. DSM 40297	-	+	-	-	-	-	-	+	-	-	+	-	+	+	+	-	-	+	+	-
<i>S. fradiae</i> DSM 40943	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+
<i>S. poonensis</i> ISP 5596	+	+	-	+	+	+	+	+	+	-	+	+	-	+	-	+	-	+	+	+
<i>Streptomyces</i> sp. DSM 40857	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+
<i>S. olivaceus</i> DSM 40756	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+
<i>S. erythrogriseus</i> ISP 5116	+	+	-	-	-	+	+	-	+	+	+	-	+	+	+	+	-	+	+	-
<i>S. bambergiensis</i> ISP 5590 ^T	+	+	+	-	-	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+
<i>S. ipomoeae</i> ISP 5383	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
<i>S. exfoliatus</i> DSM 40735	+	+	-	+	+	+	-	+	-	-	-	-	+	-	-	-	-	+	-	-
<i>S. aureofaciens</i> DSM 40643	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
<i>S. xanthophaeus</i> ISP 5134	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. hygroscopicus</i> ISP 5578	-	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+
<i>S. coelicolor</i> DSM 40674	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	+	+	-
<i>A. autotrophica</i> ISP 5011	-	+	-	-	-	-	-	+	-	-	-	-	-	-	+	+	+	+	-	+

[illegible]

Methods

Clusters. Details on the origins of strains and the composition of clusters were given by Kämpfer *et al.* (1991). The single-member clusters obtained in that study were not included in the matrix; the test results for these are given as a diagnostic table.

Some phenotypes containing related strains and showing much overlap were regarded as one group. The minor clusters 51, 53, 54, 55 and 57, containing strains of *Streptomyces violaceusniger* and *S. hygroscopicus*, were joined. Cluster 30 (*S. albus*) was grouped with clusters 31 (*S. cacaoi*) and 32 (*S. albus*), with which it showed much overlap. Similarly, clusters 35 and 36, both containing strains of *S. rimosus*, were grouped together. Names of the clusters were adopted from the study of Kämpfer *et al.* (1991).

Selection of the most diagnostic characters. Procedures for objective character selection have been described in detail by Sneath (1979*b*; 1980*a, b*). These procedures have been successfully applied to streptomycetes in the studies of Williams *et al.* (1983*b*; 1985) and Langham *et al.* (1989*a*).

The minimum number of characters was selected from the 329 characters used by Kämpfer *et al.* (1991). From the several separation indices provided by the CHARSEP program (Sneath, 1979*b*), the VSP index (variance of separation potential) was chosen to find the best differentiating characters. Fifty characters were chosen for the matrix. The selected characters were assessed using the MOSTYP program (Sneath, 1980*b*), which provides the identification scores of the Willcox probability (Willcox *et al.*, 1973), the taxonomic distance and the standard error of the taxonomic distance for the hypothetical median organism of each cluster. The DIACHAR program (Sneath, 1980*a*) was used to assess the chosen characters.

Determination of characteristics in the matrix. Each strain was tested for 50 characters (Table 1) including carbon source utilization tests and qualitative enzyme tests using chromogenic substrates. Tests were performed in standard microtitration plates (Greiner, Nürtingen, FRG). All tests were read both visually and photometrically.

All strains were cultivated on glucose/yeast extract/malt extract agar (GYM) containing the following constituents (g l⁻¹): yeast extract, 4; malt extract, 10; D-glucose, 4; CaCO₃, 2; agar, 15. They were incubated aerobically at 28 °C.

(i) **Carbon source utilization.** Growth on different carbon sources was tested in a mineral medium containing the following constituents (g l⁻¹): K₂HPO₄, 1.74; KH₂PO₄, 1.36; (NH₄)₂SO₄, 5.0; MgSO₄·7H₂O, 0.5; CaCl₂·2H₂O, 0.1; NaCl, 9.0; yeast extract (Oxoid), 0.02; bio-lactysate (bio-Merieux), 0.02; vitamin solution, 5 ml [containing (mg l⁻¹): calcium pantothenate, 0.1; nicotinic acid, 0.1; biotin, 0.005; cyanocobalamin, 0.005; folic acid, 0.1; pyridoxine, 0.1; *p*-aminobenzoic acid, 0.1; thiamin pyrophosphate, 0.1; inositol, 0.1; thiamin, 0.1 riboflavin, 0.1]; trace element solution, 1 ml [containing (mg l⁻¹): H₃BO₃, 0.5; CuSO₄·5H₂O, 0.04; KI, 0.1; FeCl₃·6H₂O, 0.2; MnSO₄·H₂O, 0.2; Na₂MoO₄·2H₂O, 0.2; ZnSO₄·7H₂O, 0.4]. After adding the different carbon sources, at a final concentration of 0.2% (w/v), the media were sterilized by filtration.

(ii) **Qualitative enzyme tests with chromogenic substrates.** These tests were performed in filter-sterilized medium (pH 7.2) containing 0.05 M-Tris/HCl buffer and 0.05% (w/v) each of yeast extract (Oxoid) and bio-lactysate (bio-Merieux). Filter-sterilized solutions of the glycosidase substrates (*p*-nitrophenyl-linked substrates) were added at final concentrations of 2 mM. For L-valine-*p*-nitroanilide a concentration of 1 mM was used.

Testing procedure. To obtain homogeneous suspensions for inoculation of the different test wells, all strains were cultivated in liquid GYM medium for 72 h at 28 °C. After growth, the cell agglomerates were mechanically homogenized using a sterile Ultraturrax (Jahnke &

Kunkel, Staufen, FRG) for 1 min at 4000 r.p.m. From this homogenate, 100 µl aliquots were transferred to 5 ml of 0.9% (w/v) NaCl and each test well was filled with 50 µl of the bacterial suspension. Test plates were covered with plastic seals (Flow Laboratories) and incubated at 28 °C for 14 d. Test results were read photometrically with a Multiscan MCC340 photometer (Flow Laboratories). A carbon substrate utilization test was considered positive if $A_{414}(\text{test}) - A_{414}(\text{assimilation control}) > 0.05$, and a qualitative enzyme test was considered positive if $A_{414}(\text{test}) - A_{414}(\text{chromogenic substrate control}) > 0.3$. Because of the different growth characteristics of the strains, all test results were also checked visually.

Theoretical evaluation of the matrix. The matrix was further evaluated using the OVERMAT program (Sneath, 1980*c*), which calculates the probability of significant overlap between the phenotypes. For each pair of groups, a disjunction index (W) and a corresponding nominal overlap (V_G) is calculated ranging from 1 for complete overlap to 0 for complete disjunction. The identification scores were calculated using the MATIDEN program (Sneath, 1979*a*), which provides several identification scores for known and unknown strains of q taxa and m characters. Percentages in the matrix, with 0 changed to 1 and 100 changed to 99, are converted to proportions P_{ij} for the i th character of taxon J . The character states of an unknown (u) are input and compared with each taxon in turn. Three identification coefficients were calculated.

1. Willcox probability (p). This is the likelihood of u against taxon J divided by the sum of the likelihoods of u against all q taxa (L_{uj}). The nearer the score approaches to 1.0, the better is the fit of an unknown with a taxon in the matrix.

2. Taxonomic distance (d), which is given by $\sqrt{[\sum(u_i - P_{ij})^2/m]}$. This expresses the distance of an unknown from the centroid of the group with which it is compared.

3. The standard error of the taxonomic distance, which is given by the constant (c) in the equation $d = \bar{d}_j + cs_{dj}$, where $\bar{d}_j$ is the mean distance of OTUs of taxon J from the centroid and s_{dj} is the standard deviation of those distances. An acceptable score is less than 2.0 to 3.0, and about the half of the members of a taxon will have negative scores, indicating a stronger relationship to the centroid as the average.

More details of these scores are given by Sneath (1979*a*) and Williams *et al.* (1983*b*).

The results of the Willcox probability p were grouped into different intervals ($p > 0.99$; $0.99 > p \geq 0.90$; $0.9 > p \geq 0.8$; $0.8 > p \geq 0.5$; $p < 0.5$), and the highest and lowest values of the taxonomic distance and the standard error were calculated for each phenon.

The MOSTYP program (Sneath, 1980*b*) calculates the identification coefficients for the hypothetical median organism of each taxon in the matrix.

Practical evaluation of the matrix. The matrix was practically evaluated with 32 strains, all of them classified using the numerical survey of Williams *et al.* (1983*a*). The test data were again assessed using the MATIDEN program.

Computing. All calculations were done on a personal computer (Compaq Deskpro 80386).

Results

Composition of the matrix

The matrix (Table 1) consisted of 52 phenotypes versus 50 tests, and included strains from both major and minor clusters obtained in the classification of Kämpfer *et al.* (1991). For single-member clusters a diagnostic table is

given (Table 2). The 50 tests which were able to differentiate between the phena (Tables 1 and 2) included 45 carbon substrate utilization tests and five qualitative enzyme tests using chromogenic substrates. Most characters showed VSP indices $>30\%$, and thus exceeded the value of $>25\%$ recommended by Sneath (1979*b*). Scores ranged from 21.26% (spermine utilization) to 67.01% (α -D-raffinose utilization). Sums of diagnostic scores produced by the DIACHAR program ranged from 11.75 (*S. rochei*) to 25.27 (*S. fragilis*). The OVERMAT program showed a total of 80 overlaps (from 1431 possible) taking a 5% level and a confidence interval of $P = 90$. For the majority of major phena containing more than 10 strains, no overlap with other phena was observed. The theoretically best identification score for the most typical member of each phenon was calculated using the MOSTTYP program. Willcox probabilities for all phena containing two or more strains exceeded 0.999. Scores for taxonomic distances ranged from 0.184 (*S. albidoflavus*) to 0.308 (*S. griseolus*). Standard errors of the taxonomic distances were negative in most cases or achieved the score 0.05 for all phena containing only two strains.

A summary of scores obtained from the calculations of the programs CHARSEP, MOSTTYP, DIACHAR and OVERMAT is given in Table 3.

Theoretical evaluation of the matrix

All 782 isolates were tested against the matrix and the identification coefficients of the MATIDEN program were calculated. The organisms were grouped into different intervals of the Willcox probabilities p obtained in these calculations (Table 4). A total of 591 strains (75.57%) were identified with $p > 0.99$, 69 strains (8.82%) were identified correctly with $p > 0.9$, and 16 strains (2.04%) with $p > 0.8$. For 86 strains (10.99%), the values for the Willcox probability ranged between 0.50 and 0.79, i.e. the correct identification result was shown, but with low

Willcox probabilities. Twenty strains (2.55%) were not correctly identified. The identification rate of the matrix (Willcox probability >0.90) was 84.39%.

Practical evaluation of the matrix

As with the theoretical evaluation, the Willcox probabilities obtained from 32 strains were assigned to different intervals. All of the isolates were grouped into the correct phenon, although some with low Willcox probabilities. Twenty isolates (62.50%) were identified with $p > 0.99$, five (15.62%) with $0.99 > p \geq 0.90$, three (9.37%) with $0.90 > p \geq 0.80$, and four (12.50%) with $p < 0.80$. Strains of the phena *S. albidoflavus*, *S. halstedii*, *S. griseus*, *S. fradiae*, *S. rimosus*, *S. albus* and *Streptovercillium griseocarneum* could be identified with Willcox probabilities > 0.999 . Taking a minimum Willcox probability of 0.90 for correct identification, a total of 25 strains (78.12%) were correctly identified.

Discussion

The use of numerical identification and the development of matrices for routine use has been successfully applied to various groups of bacteria. Large databases of physiological data are important sources of differentiating characters. The numerical studies of Williams *et al.* (1983*a*) and Kämpfer *et al.* (1991) showed that there is wide phenetic variation in the genus *Streptomyces*. Despite the agreement of both studies in the recovery of many major phena, there were differences in the composition and arrangement of several phena. Although the study of Kämpfer *et al.* (1991) included 821 strains which were tested for 329 characters, the results indicate, in comparison with the analysis of Williams *et al.* (1983*a*), that the genus *Streptomyces* is still overspeciated, and the view that species of the genus *Streptovercillium* should be included into the genus *Streptomyces* is supported.

As pointed out by Williams *et al.* (1983*b*), there is no simple, rapid procedure for objective identification of streptomycetes. Until now, there has been no alternative to the use of the probability matrices of Williams *et al.* (1983*b*) and Langham *et al.* (1989*a*) for correct identification. However, as no differentiating characters were given for the use of two probability matrices defined for major and minor clusters (Langham *et al.*, 1989*a*), the use of two systems is still laborious. The use of a single matrix, with miniaturized tests, may be a more economical and less time-consuming alternative. However, although tests may be read photometrically, the results must also be checked visually, because of the different growth characteristics of the strains.

Table 3. Summary of scores obtained in the construction and assessment of clusters containing two or more strains

Program	Information	Range of scores
CHARSEP	VSP (%)	21.26 to 67.01
MOSTTYP	Hypothetical median organism	
	Willcox probability	0.999 to 1.000
	Taxonomic distance (d)	0.184 to 0.308
	Standard error of d	-3.943 to 0.050
DIACHAR	Sum of diagnostic scores	11.75 to 25.27
OVERMAT	Number of cluster pairs	
	with $>5\%$ overlap	80

Table 4. Theoretical evaluation of the probability matrix: numbers of strains assigned to the correct phenon with different Willcox probabilities (p)

No. of cluster or subcluster	Phenon	Total no. of strains	Willcox probability (p)				
			≥ 0.99	$0.99 > p \geq 0.9$	$0.9 > p \geq 0.8$	$0.8 > p \geq 0.5$	$p < 0.5$
1-1	<i>S. albidoflavus</i>	(52)	50	1	0	1	0
1-2	<i>S. halstedii</i>	(6)	5	1	0	0	0
1-3	<i>S. griseus</i>	(81)	66	4	1	8	2
1-4	<i>S. olivaceus</i>	(6)	6	0	0	0	0
1-5	<i>S. antibioticus</i>	(19)	13	4	0	2	0
1-6	<i>S. flaveolus</i>	(16)	10	1	1	3	1
1-7	<i>S. diastatochromogenes</i>	(25)	19	2	0	3	1
2	<i>S. exfoliatus</i>	(30)	22	1	2	5	0
3	<i>S. gelaticus</i>	(3)	3	0	0	0	0
4	<i>S. graminofasciens</i>	(6)	6	0	0	0	0
5	<i>S. parvus</i>	(5)	5	0	0	0	0
6	<i>S. rochei</i>	(79)	40	13	2	20	4
7	<i>S. prasinopilosus</i>	(7)	7	0	0	0	0
8	<i>Streptomyces</i> sp.	(4)	4	0	0	0	0
9	<i>S. violaceus</i>	(118)	60	24	5	23	6
10	<i>S. sparsogenes</i>	(2)	2	0	0	0	0
11	<i>S. psammoticus</i>	(5)	3	0	1	0	1
12	<i>S. aurantiacus</i>	(2)	2	0	0	0	0
13	<i>S. niveoruber</i>	(9)	7	2	0	0	0
14	<i>Streptomyces</i> sp.	(2)	2	0	0	0	0
17	<i>S. griseoloalbus</i>	(2)	2	0	0	0	0
20	<i>S. chrysomallus</i>	(6)	5	0	0	0	1
21	<i>S. thermovulgaris</i>	(5)	4	1	0	0	0
22-1	<i>Sv. griseocarneum</i>	(51)	49	1	1	0	0
22-2	<i>S. roseoviridis</i>	(5)	4	0	0	1	0
22-3	<i>S. lavendulae</i>	(43)	29	5	1	7	1
22-4	<i>S. misakiensis</i>	(17)	13	1	1	1	1
22-5	<i>S. fradiae</i>	(17)	12	1	0	3	1
23	<i>S. griseolus</i>	(2)	2	0	0	0	0
24	<i>S. fungicidicus</i>	(4)	4	0	0	0	0
25	<i>S. lydicus</i>	(17)	12	2	0	3	0
26	<i>S. flavidovirens</i>	(2)	2	0	0	0	0
27	<i>S. fradiae</i>	(2)	2	0	0	0	0
28	<i>S. prasinus</i>	(2)	2	0	0	0	0
30	<i>S. albus</i>	(21)	19	0	1	1	0
33	<i>S. alboflavus</i>	(19)	15	1	0	3	0
35	<i>S. rimosus</i>	(26)	21	3	0	1	1
41	<i>S. endus</i>	(2)	2	0	0	0	0
44	<i>Streptomyces</i> sp.	(2)	2	0	0	0	0
49	<i>S. roseiscleroticus</i>	(2)	2	0	0	0	0
50	<i>S. violatus</i>	(2)	2	0	0	0	0
51	<i>S. violaceusniger</i>	(17)	15	1	0	1	0
64	<i>S. longissimus</i>	(3)	3	0	0	0	0
65	<i>Streptomyces</i> sp.	(2)	2	0	0	0	0
66	<i>S. flocculus</i>	(3)	3	0	0	0	0
68	<i>S. sulphureus</i>	(2)	2	0	0	0	0
69	<i>S. ochraceiscleroticus</i>	(10)	10	0	0	0	0
76	<i>Streptomyces</i> sp./ <i>Nocardia</i> sp.	(9)	9	0	0	0	0
78	<i>S. fragilis</i>	(2)	2	0	0	0	0
79	<i>S. lipmanii</i>	(3)	3	0	0	0	0
80	<i>Streptomyces</i> sp.	(3)	3	0	0	0	0
82	<i>Streptomyces</i> sp.	(2)	2	0	0	0	0
No. of strains:		(782)	591	69	16	86	20
Percentages		100	75.57	8.82	2.04	10.99	2.55

Our matrix was based on the classification of Kämpfer *et al.* (1991), which encompassed many more strains than that of Williams *et al.* (1983a), including those assigned to species category IV of Williams *et al.* (1989). The identification rate of 78.12% for 32 strains taking a Willcox probability of 0.90 as the level for correct identification was satisfactory compared with the value of 77% obtained by Langham *et al.* (1989a), who took a Willcox probability level of 0.85 for correct identification. This value is also in line with those obtained in other probabilistic systems, such as 70.8% for Gram-negative non-fermentative bacteria (Lapage *et al.*, 1973), 50.5% for some 'coryneform' bacteria (Hill *et al.*, 1978), 79.4% for vibrios (Dawson & Sneath, 1985), 89.2% for Gram-negative fermentative bacteria (Holmes *et al.*, 1986) and 70% for bacilli (Priest & Alexander, 1988).

As pointed out by Sneath (1979b), the Willcox probability is a Bayesian coefficient and the score is expressed as a proportion of the sum of the antilogarithms for all groups. It can occasionally happen that the unknown does not belong to any group of the matrix, but fits to one group better than the other alternatives, because the normalizing step $L_{uj}/\sum^q L_{uj}$ gives the first alternative a score closer to 1.0 (Sneath, 1979a). The poor fit is indicated by numerous 'character against' scores, which have to be checked, as indicated by Langham *et al.* (1989b). On the other hand, the use of the taxonomic distance and the standard error of the taxonomic distance is not deceptive, if the true group to which the unknown belongs is not in the matrix, because the scores will be large. The criteria for a successful identification are thus a Willcox probability greater than 0.90 with low scores for taxonomic distance and its standard error; and 'characters against' scores should be zero or few (Williams *et al.*, 1983b).

The problems of streptomycete identification shown by the low Willcox probabilities for several strains (Table 4) are largely a reflexion of the difficulties in streptomycete taxonomy. We did not include morphological or pigmentation characteristics, because it is generally accepted that more than one category of spore chains can be observed in the same species and the distinction between *Retinaculiaperti* and *Spirales* is not clear (Williams & Wellington, 1980; Williams *et al.*, 1989). The determination of the colour of the spore mass is also not easy (Kutzner, 1981), and especially with respect to spore colour and other morphological criteria, our study supports the view of Williams *et al.* (1983a) that these traditional characters, which are often difficult to determine (Williams & Wellington, 1980; Kutzner, 1981), are inadequate for classification and identification. The most diagnostic physiological criteria were selected as objectively as possible.

Carbon substrate utilization tests have been found in

earlier studies to be characters with great differentiation potential. The utilization of sucrose, L-arabinose, *i*-inositol, mannitol, rhamnose and raffinose were recommended as an aid to species differentiation in the International Streptomyces Project (Shirling & Gottlieb, 1966; 1968a, b; 1969, 1972) and these tests have been used in several identification schemes (Szabo *et al.*, 1975; Küster, 1972; Nonamura, 1974; Pridham & Tresner, 1974), including automated identification systems (Gyllenberg *et al.*, 1975). These tests were also used in the probability matrices of Langham *et al.* (1989a), who found that the utilization of melibiose was an additional differentiating character. The utilization of organic acids such as malonate for identification purposes was recommended by Nitsch & Kutzner (1969).

The use of qualitative enzyme tests for differentiation of streptomycetes was proposed by Goodfellow *et al.* (1987), who used growth-independent tests of the hydrolysis of methylumbelliferyl-linked substrates to differentiate between selected strains from the species and species-groups defined by Williams *et al.* (1983a).

Recently, quantitative chemotaxonomic methods have been applied to some species of the genus *Streptomyces* (O'Donnell, 1988), but the differentiation potential of quantitative fatty acid data at the species level is still questionable (Saddler *et al.*, 1987).

It must be emphasized that streptomycete taxonomy is still developing. The phenetic diversity of streptomycetes is a major problem, and is the main reason for the large number of tests needed for species identification. Despite these problems, there is no alternative to a computer-assisted, numerical identification procedure for an objective assignment of an unknown streptomycete strain to a polythetically defined species. Based on the thesis that good classification is a prerequisite for accurate identification (Hill, 1974; Goodfellow, 1986), it is still necessary to use genetical methods on a large number of strains to confirm the taxonomic position of the numerically defined species.

References

- DAWSON, C. W. & SNEATH, P. H. A. (1985). A probability matrix for identification of vibrios. *Journal of Applied Bacteriology* **58**, 407–423.
- GOODFELLOW, M. (1986). Actinomycetes systematics: present state and future prospects. In *Biological, Biochemical and Biomedical Aspects of Actinomycetes*, Part B, pp. 487–496. Edited by G. Szabo, S. Biro & M. Goodfellow. Budapest: Akademiai Kiado.
- GOODFELLOW, M., LONSDALE, C., JAMES, A. L. & McNAMARA, O. C. (1987). Rapid biochemical tests for the characterisation of streptomycetes. *FEMS Microbiology Letters* **43**, 39–44.
- GYLLENBERG, H. G., NIEMELÄ, T. K. & NIEMI, S. J. (1975). A model for automated identification of streptomycetes. *Postepy Higieny i Medycyny Doswiadczalnej* **29**, 357–383.
- HILL, L. R. (1974). Theoretical aspects of numerical identification. *International Journal of Systematic Bacteriology* **24**, 494–499.

- HILL, L. R., LAPAGE, S. P. & BOWIE, I. S. (1978). Computer assisted identification of coryneform bacteria. In *Coryneform Bacteria*, pp. 181–215. Edited by I. J. Bousfield & A. G. Calley. London: Academic Press.
- HOLMES, B., PINNING, C. A. & DAWSON, C. A. (1986). A probability matrix for the identification of Gram-negative, aerobic, non-fermentative bacteria that grow on nutrient agar. *Journal of General Microbiology* **132**, 1827–1842.
- KÄMPFER, P., KROPPESTEDT, R. M. & DOTT, W. (1991). A numerical classification of the genera *Streptomyces* and *Streptoverticillium* using miniaturized physiological tests. *Journal of General Microbiology* **137**, 1831–1891.
- KÜSTER, E. (1972). Simple working key for the classification and identification of named taxa included in the International Streptomyces Project. *International Journal of Systematic Bacteriology* **22**, 139–148.
- KURYLOWICZ, W., PASZKIEWICZ, A., WOZNICKA, W., KURZATKOWSKI, W. & SZULGA, T. (1975). Classification of *Streptomyces* by different numerical methods. *Postępy Higieny i Medycyny Doswiadczalnej* **29**, 281–355.
- KUTZNER, H. J. (1981). *Streptomyces*. In *The Prokaryotes: a Handbook of Habitats, Isolation and Identification of Bacteria*, pp. 2028–2090. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer Verlag.
- LANGHAM, C. D., WILLIAMS, S. T., SNEATH, P. H. A. & MORTIMER, A. M. (1989a). New probability matrices for identification of *Streptomyces*. *Journal of General Microbiology* **135**, 121–133.
- LANGHAM, C. D., SNEATH, P. H. A., WILLIAMS, S. T. & MORTIMER, A. M. (1989b). Detecting aberrant strains in bacterial groups as an aid to constructing databases for computer identification. *Journal of Applied Bacteriology* **66**, 339–352.
- LAPAGE, S. P., BASCOMB, B., WILLCOX, W. R. & CURTIS, M. A. (1973). Identification of bacteria by computer: general aspects and perspectives. *Journal of General Microbiology* **77**, 273–299.
- LOCCI, R. & SCHOFIELD, G. M. (1989). Genus *Streptoverticillium* Baldacci 1958, 15, emend. mut. char. Baldacci, Farina and Locci 1966, 168^{AL}. In *Bergey's Manual of Systematic Bacteriology*, pp. 2492–2504. Edited by S. T. Williams, M. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- LOCCI, R., ROGERS, J., SARDI, G. & SCHOFIELD, G. M. (1981). A preliminary numerical study on named species of the genus *Streptoverticillium*. *Annali di Microbiologia* **31**, 115–121.
- NITSCH, B. & KUTZNER, H. J. (1969). Decomposition of oxalic acid and other organic acids by streptomycetes as a taxonomic aid. *Zeitschrift für Allgemeine Mikrobiologie* **9**, 613–632.
- NONAMURA, H. (1974). Key for classification and identification of 458 species of the streptomycetes included in ISP. *Journal of Fermentation Technology* **52**, 78–92.
- O'DONNELL, A. (1988). Recognition of novel actinomycetes. In *Actinomycetes in Biotechnology*, pp. 69–88. Edited by M. Goodfellow, S. T. Williams & M. Mordarski. London: Academic Press.
- PRIDHAM, T. G. & TRESNER, H. D. (1974). *Streptomyces* Waksman and Henrici 1943, 339. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 748–829. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- PRIEST, F. G. & ALEXANDER, B. (1988). A frequency matrix for probabilistic identification of some bacilli. *Journal of General Microbiology* **134**, 3011–3018.
- SADDLER, G. S., O'DONNELL, A. G., GOODFELLOW, M. & MINNIKIN, D. E. (1987). SIMCA pattern recognition in the analysis of streptomycete fatty acids. *Journal of General Microbiology* **133**, 1137–1147.
- SHIRLING, E. B. & GOTTLIEB, D. (1966). Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology* **16**, 313–340.
- SHIRLING, E. B. & GOTTLIEB, D. (1968a). Cooperative description of type cultures of *Streptomyces*. II. Species descriptions from first study. *International Journal of Systematic Bacteriology* **18**, 69–118.
- SHIRLING, E. B. & GOTTLIEB, D. (1968b). Cooperative description of type cultures of *Streptomyces*. III. Additional species descriptions from first and second studies. *International Journal of Systematic Bacteriology* **18**, 279–391.
- SHIRLING, E. B. & GOTTLIEB, D. (1969). Cooperative description of type cultures of *Streptomyces*. IV. Species descriptions from the second, third and fourth studies. *International Journal of Systematic Bacteriology* **19**, 391–512.
- SHIRLING, E. B. & GOTTLIEB, D. (1972). Cooperative description of type cultures of *Streptomyces*. V. Additional descriptions. *International Journal of Systematic Bacteriology* **22**, 265–394.
- SNEATH, P. H. A. (1974). Test reproducibility in relation to identification. *International Journal of Systematic Bacteriology* **24**, 508–523.
- SNEATH, P. H. A. (1979a). BASIC program for identification of an unknown with presence-absence data against an identification matrix of percent positive characters. *Computers and Geosciences* **5**, 195–213.
- SNEATH, P. H. A. (1979b). BASIC program for character separation indices from an identification matrix of percent positive characters. *Computers and Geosciences* **5**, 349–357.
- SNEATH, P. H. A. (1980a). BASIC program for the most diagnostic properties of groups from an identification matrix of percent positive characters. *Computers and Geosciences* **6**, 21–26.
- SNEATH, P. H. A. (1980b). BASIC program for determining the best identification scores possible for the most typical example when compared with an identification matrix of percent positive characters. *Computers and Geosciences* **6**, 27–34.
- SNEATH, P. H. A. (1980c). BASIC program for determining overlap between groups in an identification matrix of percent positive characters. *Computers and Geosciences* **6**, 267–278.
- SZABO, I. M., MARTON, M., BUTI, I. & FERNANDEZ, C. (1975). A diagnostic key for the identification of 'species' of *Streptomyces* and *Streptoverticillium* included in the International Streptomyces Project. *Acta Botanica Academiae Scientiarum Hungaricae* **21**, 387–418.
- WILLCOX, W. R., LAPAGE, S. P., BASCOMB, S. & CURTIS, M. A. (1973). Identification of bacteria by computer: theory and programming. *Journal of General Microbiology* **77**, 317–330.
- WILLIAMS, S. T. & WELLINGTON, E. M. H. (1980). Micromorphology and fine structure of actinomycetes. In *Microbiological Classification and Identification*, pp. 139–165. Edited by M. Goodfellow & R. G. Board. London: Academic Press.
- WILLIAMS, S. T., GOODFELLOW, M., ALDERSON, G., WELLINGTON, E. M. H., SNEATH, P. H. A. & SACKIN, M. J. (1983a). Numerical classification of *Streptomyces* and related genera. *Journal of General Microbiology* **129**, 1743–1813.
- WILLIAMS, S. T., GOODFELLOW, M., WELLINGTON, E. M. H., VICKERS, J. C., ALDERSON, G., SNEATH, P. H. A., SACKIN, M. J. & MORTIMER, A. M. (1983b). A probability matrix for identification of some streptomycetes. *Journal of General Microbiology* **129**, 1815–1830.
- WILLIAMS, S. T., LOCCI, R., VICKERS, J. C., SCHOFIELD, G. M., SNEATH, P. H. A. & MORTIMER, A. M. (1985). Probabilistic identification of *Streptoverticillium* species. *Journal of General Microbiology* **131**, 1881–1889.
- WILLIAMS, S. T., GOODFELLOW, M. & ALDERSON, G. (1989). Genus *Streptomyces* (Waksman & Henrici 1943) 339^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2452–2492. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.