Jurnal Mikrobiologi Indonesia, Februari 2001, hlm. 1-7
ISSN 0853-358X

Vol. 6, No. 1

Ulas Balik

Application of Numerical Systematics in Unraveling Streptomycete Diversity

LANGKAH SEMBIRING[1]* & MICHAEL GOODFELLOW[2]

[1]*Laboratorium Mikrobiologi, Fakultas Biologi, Universitas Gadjah Mada, Jalan Teknika Selatan, Sekip Utara, Kampus Bulaksumur, Yogyakarta 55281*
[2]*Department of Agricultural and Environmental Science, University of Newcastle, Newcastle upon Tyne, NE1 7RU, UK*

Systematics plays an important role in the utilization of streptomycete strains as an outstanding producer of thousands documented bioactive compounds. The chaotic state of streptomycete systematics resulted from the application of traditional monothetic approach had clearly hampered the progress of development of reliable identification system for potential streptomycete strains. However, the extensive application of polythetic numerical method by increasing number of experts was proved to be successful in developing a more sounding streptomycete classification system. As a consequence, such classification system can subsequently be used as a rigorous basis to generate a more reliable identification system in attempt to unravel the extent of streptomycete diversity in natural habitats both at the inter and intraspecies level. This review addresses the problem and the role of numerical systematics in the development of current status of streptomycete systematics which is applicable to delimit species within the genus.

Key words: streptomycete systematics, monothetic, polythetic, streptomycete diversity

Streptomycete systematics has had a long and tortuous history (Goodfellow *et al.* 1992, Korn-Wendisch & Kutzner 1992, Manfio 1995). Early descriptions of streptomycete species by soil microbiologists were based on ecological requirements, pigmentation and spore chain morphology (Krainsky 1914, Conn 1916, Waksman & Curtis 1916, Waksman 1919, Jensen 1930) and dichotomous keys for the identification of unknown strains rested on a few non-standardised tests, notably morphological and pigmentation characteristics (Krainsky 1914, Waksman & Curtis 1916, Waksman 1919, Jensen 1930, Krassilnikov 1941). A turning point in the systematics of the taxon came in 1943 when Waksman & Henrici proposed the genus *Streptomyces* (Strep.to.my'ces. Gr. adj. *streptos* pliant, bent; Gr. n: *myces* fungus; M.L. masc. n.: *Streptomyces* pliant or bent fungus) for aerobic spore-forming actinomycetes to avoid confusion with pathogenic microaerophilic organisms which retained the name *Actinomyces* Harz 1877.

It was only after the discovery that *Streptomyces antibioticus* produced actinomycin (Waksman & Woodruff 1941) that widespread interest was taken in the genus. The realization that streptomycetes were a rich source of commercially useful antibiotics led many workers to design new procedures for their isolation and growth. Lack of acceptable criteria for classification and identification led to new species being described usually on the basis of slight differences in morphological and cultural properties. This practice, associated with difficulties of identification due to poor classification, led to a proliferation of *Streptomyces* species (Waksman 1957, Kurylowicz & Gyllenberg 1988). Between 1940 and 1957 over a hundred *Streptomyces* species were described (Pridham *et al.* 1958). This number increased to around 3 000 by 1970 though many of the new combinations were cited only in the patent literature (Trejo 1970). Numerous artificial classifications were devised to accommodate the ever increasing number of *Streptomyces* species. These classifications were based mainly on a few subjectively chosen characters, usually morphological and pigmentation properties, though in some instances biochemical, nutritional and physiological features were used (Table 1). These schemes enabled isolates to be "identified" but the resultant names were dependent on the scheme used.

Chaotic Period of Streptomycete Systematics. It was clear by the early 1960's that streptomycete systematics was in a chaotic state. The resultant practical problems were addressed in two co-operative investigations carried out between 1958 and 1962. One of the studies was performed under the auspices of the *Subcommittee on Actinomycetes* of the *Committee on Taxonomy of the American Society of Microbiology* (ASM, Gottlieb 1961) and the other by the *Subcommittee on Taxonomy of Actinomycetes* of the *International Committee on Bacteriological Nomenclature of the International Association of Microbiological Societies* (IAMS, Küster 1959).

An attempt was made in each of the cooperative studies to evaluate the predictiveness of characters commonly used in streptomycete classification and identification. In the IAMS

* Penulis untuk korespondensi, Tel. +62-274-543631, Fax. +62-274-580839, E-mail: lsembiring@yahoo.com

Table 1. Criteria used in early classifications of streptomycetes*

Criteria used and their priorities**					No. of groups	References
Arrangement of spores in chains	Aerial spore mass colour	Substrate mycelium colour	Spore surface ornamentation	Production of melanin pigments		
2	1				7	Hesseltine *et al.* (1954)
	1	2			15	Gauze *et al.* (1957)
3	2		1	4	34	Ettlinger *et al.* (1958)
1	2				42	Pridham *et al.* (1958)
3	2	1			33	Baldacci (1958)
	1	2			10	Flaig & Kutzner (1960)
	2	3		1	16	Waksman (1961)
3	2		1	4	41	Hütter (1967)

*Modified from Williams *et al.* (1989).
**Numbers under each character indicate the order of its priority in the classification of streptomycetes used by the corresponding author (s).

study, 34 investigators examined 25 strains, representing 21 streptomycete "series", using standard methods and growth conditions (Küster 1959). The findings of the study were published (Küster 1961, Szabó & Marton 1964). Ten laboratories were involved in the corresponding ASM collaborative project which concluded that more work was required before reliable physiological tests could be recommended for streptomycete systematics (Gottlieb 1961).

The results of the two collaborative studies showed that developments in streptomycete systematics were being hampered by the use of variable and non-diagnostic characters which were often examined under non-standardized conditions. The reliance placed on subjectively weighted phenotypic characters, the unavailability of extant type cultures of some species and the difficulty in finding descriptions of species reported in the patent literature were all seen to be serious problems. The cooperative projects raised as many problems as they answered, but they did pave the way for an extensive international collaborative study on the genus *Streptomyces*.

The International *Streptomyces* Project (ISP, Shirling & Gottlieb 1966) was planned and carried out by the *Subcommittee of Taxonomy of Actinomycetes* of the *International Committee on Bacteriological Nomenclature* and the *Subcommittee on Actinomycetes* of *the Committee on the Taxonomy of the American Society of Microbiology* with the primary aim of providing reliable descriptions of extant and authentic type strains of *Streptomyces* and *Streptoverticillium* species. Existing type and neotype strains of species assigned to these genera were sent under code to at least three experts in different countries. The strains were examined using rigorously standardized procedures to determine their morphological, pigmentation and carbon source utilization properties. These characters were selected in light of the results from the earlier international co-operatives studies (Küster 1959, Gottlieb 1961, 1963). The methods and new descriptions of the cultures were published (Shirling & Gottlieb 1966, 1968a, 1968b, 1969, 1972, Gottlieb & Shirling 1967) and the type strains deposited in a number of internationally recognized service culture collections. The results of the International *Streptomyces* Project formed the basis of the classification of the genus *Streptomyces* in the eighth edition of *Bergey's Manual of Determinative Bacteriology* (Pridham & Tresner 1974a, 1974b).

The results of the International *Streptomyces* Project represented a major contribution to streptomycete systematics as the practical problems outlined above were met. However, the very success of the project highlighted a number of weaknesses: (i) no attempt had been made to detect synonyms or to devise a species concept for the genus (ii) few criteria were used to describe species and the ones that were applied were essentially those which had been intuitively selected from earlier classifications (Krainsky 1914, Waksman & Curtis 1916, Waksman 1919, 1961, Jensen 1930, Waksman & Henrici 1948, Baldacci *et al.* 1954, Hesseltine *et al.* 1954, Gauze *et al.* 1957, Pridham *et al.* 1958, Mayama 1959, Nomi 1960, Küster 1961, Gottlieb 1963, Hütter 1967) (iii) an objective identification system was not produced although ISP data were used to generate several dichotomous keys (Arai & Mikami 1969, Küster 1972, Nonomura 1974, Szabó *et al.* 1975) though none of them were used by other workers.

The reliance placed on a limited number of intuitively chosen features, with heavy emphasis on morphology and pigmentation, represented a serious conceptual flaw in streptomycete systematics. The products of this approach to classification are intrinsically artificial and although some of them 'work' in the sense that a name is inevitably obtained for an unknown culture, they are essentially monothetic with rigid key characters and a limited information content. It was only with the application of the numerical taxonomic procedure that attempts were made to construct polythetic classifications where organisms which share many features in common are grouped together with no single character being essential for group membership (Williams *et al.* 1981, Goodfellow *et al.* 1992).

The Application of Numerical Systematics. The numerical taxonomic procedure was first applied to representatives of the genus *Streptomyces* by Silvestri and his colleagues (Gilardi *et al.* 1960, Hill *et al.* 1961, Silvestri *et al.* 1962) who examined 200 mesophilic strains for 100 unit characters. The strains were assigned to 25 centres of taxonomic variation though some with the same specific epithet fell into different clusters. Several physiological and biochemical characters highlighted in the study were used to construct identification keys (Hill & Silvestri 1962). However, results from factor analyses suggested that many of the phenotypic characters used to describe *Streptomyces* species were highly variable and

prone to errors of interpretation (Gyllenberg 1970). These early studies had little impact on developments in *Streptomyces* systematics though several other workers applied numerical taxonomic techniques to relatively narrow databases (Table 2).

Sneath (1970) considered that a rigorous application of the numerical taxonomic procedure provided the only way of reclassifying the six-hundred "species" of the genus since reliance on a few selected tests could not be expected to reveal natural phenetic groups. The first comprehensive taxonomic survey of the genus *Streptomyces* was carried out by Williams *et al.* (1983a) who examined 475 strains, including 394 *Streptomyces* type strains from the International *Streptomyces* Project, additional type strains from related genera and some environmental isolates for 139 unit characters. The data were analysed using the Jaccard (S_J) and simple matching (S_{SM}) coefficients and the unweighted pair group method with arithmetic averages algorithm (UPGMA). The unit characters included traditional criteria used in previous studies and data from biochemical, degradative, nutritional and tolerance tests, some of which had not previously been used in streptomycete systematics.

The resultant classification added to a wealth of evidence that eventually led to the genera *Actinopycnidium* Krassilnikov 1962, *Actinosporangium* Krassilnikov & Yuan 1961, *Chainia* Thirumalachar 1955, *Elytrosporangium* Falcão de Morais *et al.*1966, *Kitasatoa* Matsumae *et al.* 1968, *Microellobosporia* Cross *et al.* 1963 and *Streptoverticillium* Baldacci 1958 becoming synonyms of the genus *Streptomyces* (Goodfellow *et al.* 1986a, 1986b, 1986c, 1986d, Witt & Stackebrandt 1990). The type strains of the *Streptomyces* species were assigned to 19 major clusters (6 to 71 strains), which were equated with species-groups, and 40 minor (2 to 5 strains) and 18 single-membered clusters that were considered to correspond to species. The clusters were named, where possible, after the earliest validly described species they contained. The results of this study forms the core of the classification of the genus *Streptomyces* in the current edition of *Bergey's Manual of Systematic Bacteriology* (Williams *et al.* 1989).

The numerical classification of Williams *et al.* (1983a) was used to generate probabilistic schemes for the identification of unknown mesophilic streptomycetes to major and minor clusters (Williams *et al.* 1983b, Langham *et al.* 1989). The computer assisted approach to the identification of unknown streptomycetes rested on a balanced set of *a posteriori* weighted characters that accommodated some degree of strain variation. This identification strategy was in sharp contrast to previous streptomycete identification schemes that were based on a few subjectively chosen features (Pridham *et al.* 1958, Waksman 1961, Hütter 1967).

Goodfellow *et al.* (1992) re-examined most of the strains studied by Williams and his colleagues for all but two of the original 139 unit characters together with the results of rapid enzyme tests based on the fluorophores 7-amino-methylcoumarin and 4-methylumbelliferone. Excellent congruence was found with the earlier numerical classification though three taxa previously defined as subclusters, namely *Streptomyces albidoflavus, Streptomyces anulatus* and *Streptomyces halstedii*, were recovered as separate, albeit related clusters.

Table 2. Numerical taxonomic studies applied to the genus *Streptomyces*.

Study	Number of strains	Number of features	Statistics	Number of clusters
Gilardi *et al.* (1960)	69	91	S_J, correlated features	5
Hill *et al.* (1961)	69	91	S_{SM}, single linkage algorithm	21
Silvestri *et al.* (1962)	159	105	S_{SM}, taxonomic distance	24
Woznicka (1965)	55	74	S-index Wroclaw dendrite	4
Gyllenberg *et al.* (1967)	60	33	Phenetic classification, factor analysis	5
Kurylowicz *et al.* (1969)	150	35	Similarity measure not given, taxonomic tree	NS*
Williams *et al.* (1969)	18	46	S_{SM}, single linkage	5
Gyllenberg (1970)	174	37	Factor analysis	NS
Kurylowicz *et al.* (1970)	150	143	S_{SM}, taxonomic tree	NS
Paskiewicz (1972)	300	200	Wroclaw dendrite	3
	300	7	Centrifugal correlation	12
Kurylowicz *et al.* (1975)	448	31	Wroclaw dendrite	14
	448	23	Centrifugal correlation	21
	448	31	S_{SM}, single linkage	14
Gyllenberg *et al.* (1975)	559	24	Q index, non-hierarchical clustering	15
Szulga (1978)	33	11	Average linkage	4
	33	11	Single linkage	3
	33	11	Principal component analysis	NS
	33	11	Sequential dendrite method	NS
Konev & Minitskii (1980)	42	44	Total mutual similarity	NS
Williams *et al.* (1983a)	394	139	S_{SM}, S_J, average linkage	3 major 5 minor 25 SMC
Goodfellow *et al.* (1987)	251	135	S_{SM}, S_J, D_p, average linkage	3 major 5 minor 2 SMC
Saddler (1988)	170	136	S_J, average linkage	9 major 18 minor 53 SMC
Kämpfer *et al.* (1991)	821	329	S_{SM}, S_J, average linkage	15 major 34 minor 40 SMC
Doering-Saad *et al.* (1992)	47	329	S_{SM}, average linkage	3 cluster groups
Goodfellow *et al.* (1992)	272	273	S_J, average linkage	23 major 20 minor 25 SMC
Sahin (1995)	159	216	S_{SM}, average linkage	12 major 14 minor 13 SMC

NS: clusters not formally defined, SMC: single-membered cluster.

It is also encouraging that most of the major clusters defined by Williams *et al.* (1983a) were recognised by Kämpfer *et al.* (1991) who examined 821 *Streptomyces* (including *Streptoverticillium* spp.) for 339 physiological tests in a comprehensive numerical taxonomic study. Kämpfer and his colleagues concluded that the taxonomic status of many of their clusters, notably the minor and single-membered clusters, were

questionable. Nevertheless, the results of their numerical taxonomic study were used to construct a probability matrix for the numerical identification of streptomycetes (Kämpfer & Kroppenstedt 1991).

Doering-Saad *et al.* (1992) examined eighty *Streptomyces* isolates, including 35 potato scab-inducing strains and 12 reference strains of *Streptomyces scabies*, for 329 unit characters. The strains were assigned to three cluster-groups (A to C) defined at the 80% similarity level in a S_{SM}, UPGMA analysis. Cluster-group A contained organisms that were related to either *Streptomyces exfoliatus* or *Streptomyces griseus* and cluster-group B encompassed strains which showed affinities to either *Streptomyces rochei* or *Streptomyces violaceus*. The majority of the pathogenic isolates and the reference strains assigned to cluster-group C were classified as either *Streptomyces griseus* or *Streptomyces violaceus*.

The first comprehensive numerical taxonomic study of thermophilic streptomycetes was carried out by Goodfellow *et al.* (1987). These workers examined fifty thermophilic neutrophilic streptomycetes from diverse habitats and compared the results with corresponding data on representative mesophilic neutrophilic marker strains that had been included in the extensive numerical taxonomic survey of Williams *et al.* (1983a). The thermophilic strains, which were grown at 45°C, were examined for one hundred and thirty five unit characters and the resultant data analysed using appropriate resemblance coefficients and clustering algorithms. Two aggregate clusters were detected, one contained the mesophilic streptomycetes and the other the thermophilic strains. The latter were assigned to two major (7 to 19 strains), four minor (2 to 3 strains) and two single-membered clusters. Three of these taxa were equated with validly described species, namely, *Streptomyces megasporus* (Krassilnikov *et al.* 1968) Agre 1983 *Streptomyces thermoviolaceus* Henssen 1957 and *Streptomyces thermovulgaris* Henssen 1957. The remaining cluster was raised to species status as *Streptomyces thermolineatus* Goodfellow *et al.* 1987.

Fifty four thermophilic carboxydotrophic actinomycetes, isolated from soils and composts, were the subject of an extensive numerical phenetic survey together with representative mesophilic and thermophilic streptomycetes (O'Donnell *et al.* 1993). The test strains, which were grown at either 25°C (mesophilic strains) or 45°C (thermophilic strains), were examined for 119 unit characters and the data analyzed using the D_p, S_j and S_{SM} coefficients and the UPGMA algorithm. The carboxydotrophic actinomycetes formed two major cluster groups which were distinct from corresponding taxa equated with mesophilic and thermophilic streptomycetes. Most of the carboxydotrophic strains grew at 55°C and all but two of them had a profile of chemical properties consistent with their assignment to the genus *Streptomyces*.

Saddler (1988) isolated large numbers of alkalitolerant mesophilic streptomycetes from a range of soils using isolation media adjusted to pH 10.0. An artificial classification of 731 alkalitolerant isolates based on pH requirements for growth, morphology and pigmentation properties revealed that 80% of the taxonomically diverse strains grew at pH 7.0 and pH 10.0. One hundred and seventy representatives of the 25 colour-groups recognized by Saddler were compared with 36 marker neutrophilic strains of *Streptomyces* species for 136 unit characters and the resultant data examined using standard numerical taxonomic procedures. The test strains were assigned to eight multi-membered and seven single-membered aggregate groups in the S_j, UPGMA analysis. The aggregate groups encompassed nine major (5 to 36 strains), eighteen minor (2 to 4 strains) and fifty three single-membered clusters. The alkalitolerant isolates were largely distinct from the *Streptomyces* marker strains. There was considerable correlation between cluster group membership and the source, colour group and pH ranges of the strains.

Sahin (1995) isolated large numbers of thermophilic streptomycetes from arid and tropical soil samples by incubating starch-casein agar plates supplemented with cycloheximide and rifampicin, and adjusted to pH 7.0 or pH 10.5, at 55°C for 5 days. Forty five alkalitolerant thermophilic streptomycetes, and eighty five neutrophilic thermophilic streptomycetes were chosen to represent groups based on aerial spore mass colour, susbstrate mycelial pigmentation, diffusible pigment colour and on the production of melanin pigments. These organisms were examined with thirty two marker neutrophilic thermophilic streptomycetes for three hundred and thirty nine unit characters together with three alkalitolerant mesophilic organisms. Eighteen randomly chosen duplicated cultures were studied under code to determine test error. A broad range of degradative, enzymatic, morphological, nutritional and physiological tests were performed to avoid undue emphasis on any particular character set. The enzymatic tests were carried out using an automated system that involved the use of conjugated substrates based on the fluorophores 7-amino-4-methylcoumarin and 4 methylumbelliferone. Fifty-six unit characters were deleted from the raw data base as they gave all positive or all negative results and a further twenty-three properties were removed because of high test error. The final data base contained information on one hundred and fifty-nine test strains and two hundred and sixty unit characters.

Good congruence was found between the classifications based on the standard resemblance coefficients (S_j, S_p, and S_{SM}) and the single linkage and UPGMA clustering algorithms. The S_{SM}, UPGMA analysis was used as the base line classification as it gave particularly good resolution of aggregate groups and clusters and a high co-phenetic correlation value; six aggregate groups encompassed twelve major (5 to 15 strains), fourteen minor (2 to 4 strains) and thirteen single-membered clusters. Cluster composition was only marginally affected by the statistics used or by the test error of 1.8%.

Thirty out of the forty-five alkalitolerant thermophilic isolates were assigned to three major (6 to 13 strains), one minor and three single-membered clusters in aggregate group VI.

One of major clusters in aggregate VI was identified as *Streptomyces thermovulgaris* as it contained the type strain of this species. The remaining fifteen alkalitolerant thermophilic isolates were assigned to one major, one minor and one single-membered cluster in aggregate group VI and to one single-membered cluster in aggregate group V.

Sixty-one out of the eighty-five neutrophilic thermophilic streptomycetes were recovered in aggregate group VI which encompassed six major (6 to 15 strains), six minor (2 to 4 strains) and three single-membered clusters. The two marker strains assigned to this aggregate group, *Streptomyces megasporus* K45^T and *Streptomyces thermolineatus* DSM 41451^T, also formed single-membered clusters. Twelve neutrophilic thermophilic isolates formed two putatively novel taxospecies that were assigned to aggregate cluster II together with two representatives of *Streptomyces albus*. The remaining twelve neutrophilic thermophilic isolates were assigned to one major (10 strains), one minor and one single-membered cluster in aggregate group VI.

The remaining aggregate taxa were composed solely of marker strains. *Streptomyces canescens* DSM 40001^T, *Streptomyces cavourensis* subspecies *cavourensis* DSM 40300^T and *Streptomyces hydrogenans* DSM 40586^T were recovered as single- membered clusters in aggregate group III and the two minor clusters which formed aggregate group V corresponded to the validly described species *Streptomyces thermodiastaticus* and *Streptomyces thermoviolaceus*. Aggregate group I contained the two marker strains of *Streptomyces megasporus*.

Finally, it can be further demonstrated that numerical taxonomy is very powerful in the classification of stretomycete isolated from the environmental samples. Several streptomycete isolates associated with the roots of tropical legume, *Paraserianthes falcataria,* notably which produce rugose ornamented spore chain could be assigned and described very well to species level by the application of numerical and molecular systematics (Sembiring *et al.* 2000).

Concluding Remark. The extensive numerical phenetic analyses considered above were partly designed to help determine the extent of streptomycete diversity in natural habitats and to provide a framework for further developments in streptomycete systematics. It was, of course, recognized by the investigators that relationships depicted in numerical classifications can be influenced by test and strain selection, test error and genetic instability of the test strains (Goodfellow & O'Donnell 1993, Schrempf *et al.* 1989, 1994) hence the need to evaluate numerical classifications in light of data derived from independent taxonomic methods, notably molecular systematic procedures.

REFERENCES

Arai T, Mikami Y. 1969. Identification keys for antibiotic producing *Streptomyces*. I. Anti fungal antibiotics producers. *Ann Rep Inst Food Microbiol* (Chiba Univ) 22:59-79.

Baldacci E. 1958. Development in the classification of actinomycetes. *Giornale Microbiol* 6:10-27.

Baldacci E, Spalla C, Grein A. 1954. The classification of the *Actinomyces* species (= *Streptomyces*). *Archiv Mikrobiol* 20:347-357.

Conn HJ. 1916. A possible function of actinomycetes in soil. *J Bacteriol* 1:191-207.

Doering-Saad C, Kampfer P, Manulis S, Kritzman G, Schneider J, Zakrzewska-Czerwinska J, Schrempf H, Barash I. 1992. Diversity among *Streptomyces* strains causing potato scab. *Appl Environ Microbiol* 58:3932-3940.

Ettlinger L, Corbaz R, Hütter R. 1958. Zür Systematik der Actinomyceten. 4. Eine Arteinteilung de Gattung *Streptomyces* Waksman et Henrici. *Archiv Mikrobiol* 31:326-358.

Flaig W, Kutzner HJ. 1960. Beitrag zur Systematik der gattung *Streptomyces* Waksman et Henrici. *Archiv Mikrobiol* 35:105-138.

Gauze GF, Preobrazhenkaya TP, Kudrina ES, Blinov NO, Ryabova ID, Sveshnikova MA. 1957. *Problems of Classification of Actinomycetes Antagonists* [dalam bahasa Rusia] Moscow: Government Publishing House of Medical Literature.

Gilardi E, Hill LR, Turri M, Silvestri L. 1960. Quantitative methods in the systematics of *Actinomycetales*. I. *Giornale Microbiol* 8:203-218.

Goodfellow M, Ferguson EV, Sanglier JJ. 1992. Numerical classification and identification of *Streptomyces* species [Ulas Balik]. *Gene* 115:225-233.

Goodfellow M, Lacey J, Todd C. 1987. Numerical classification of thermophilic streptomycetes. *J Gen Microbiol* 133:3135-3149.

Goodfellow M, O'Donnell AG. 1993. Root of bacterial systematics. Di dalam: Goodfellow M, O'Donnell AG (ed). *Handbook of New Bacterial Systematics*. London: Academic Pr. hlm 3-54.

Goodfellow M, Williams ST, Alderson G. 1986a. Transfer of *Elytrosporangium brasiliense* Falcão de Morais *et al.*, *Elytrosporangium carpinense* Falcão de Morais *et al.*, *Elytrosporangium spirale* Falcão de Morais *et al.*, *Microellobosporia cinerea* Cross *et al.*, *Microellobosporia flavea* Cross *et al.*, *Microellobosporia grisea* (Konev *et al.*) Pridham and *Microellobosporia violacea* (Tsyganov *et al.*) Pridham to the genus *Streptomyces*, with emended description of the species. *Syst Appl Microbiol* 8:48-54.

Goodfellow M, Williams ST, Alderson G. 1986b. Transfer of *Chainia* species to the genus *Streptomyces* with emended description of species. *Syst Appl Microbiol* 8:55-60.

Goodfellow M, Williams ST, Alderson G. 1986c. Transfer of *Actinosporangium violaceum* Krasilnikov & Yuan, *Actinosporangium vitaminophilum* Shomura *et al.* and descriptions of the species. *Syst Appl Microbiol* 8:61-64.

Goodfellow M, Williams ST, Alderson G. 1986d. Transfer of *Kitasatoa purpurea* Matsumae & Hata to the genus *Streptomyces* as *Streptomyces purpureus* comb. nov. *Syst Appl Microbiol* 8:65-66.

Gottlieb D. 1961. An evaluation of criteria and procedures used in the description and characterisation of the streptomycetes. A co-operative study. *Appl Microbiol* 9:55-65.

Gottlieb D. 1963. Reccommendations for description of some *Actinomycetales* appearing in patent applications. *Int Bull Bacteriol Nomenclature Taxon* 13:159-160.

Gottlieb D, Shirling EB. 1967. Cooperative description of type cultures of *Streptomyces*. I. The International *Streptomyces* Project. *Int J Syst Bacteriol* 17:315-322.

Gyllenberg HG. 1970. Factor analytical evaluation of patterns of correlated characteristics in streptomycete. Di dalam: Prauser H (ed.), *The Actinomycetales*. Jena: Veb Gustav Fischer verlag. hlm 101-105.

Gyllenberg HG, Niemella TK, Niemi JS. 1975. A model for automatic identification of streptomycetes. *Postepy Higieni i Medycyny Doswiadezalnej* [dalam bahasa Polandia] 29:357-383.

Gyllenberg HG, Woznicka W, Kurylowicz W. 1967. Application of factor analysis in microbiology, III. A study of the "yellow series" of streptomycetes. *Ann Acad Sci Fenn. Series A. IV. Biologica* 114: 3-15.

Hesseltine CW, Bennedict RG, Pridham TG. 1954. Useful criteria for species differentiation in the genus *Streptomyces*. *Ann New York Acad Sci* 60:136-151.

Hill LR, Silvestri LG. 1962. Quantitative methods in the systematics of *Actinomycetales* III. The taxonomic significance of physiological biochemical characters and the construction of a diagnostic key. *Giornale Microbiol* 10:1-28.

Hill LR, Turri M, Gilardi E, Silvestri L. 1961. Quantitative methods in the Systematics of *Actinomycetales*, II. *Giornale Microbiol* 9:56-72.

Hütter R. 1967. *Systematik der Streptomyceten onter Besonderer Berucksichtigung der von Ihnen Gebildeten Antibiotica*. Basel: S. Karger.

Jensen HL. 1930. Actinomycetes in Danish soil. *Soil Sci* 30:59-77.

Kämpfer P, Kroppenstedt RM. 1991. Probabilistic identification of strepto-mycetes using miniaturised physiological tests. *J Gen Microbiol* 137:1893-1902.

Kämpfer P, Kroppenstedtz RM, Dott W. 1991. A numerical classification of the genera *Streptomyces* and *Streptoverticillium* using miniaturized physiological tsts. *J Gen Microbiol* 137:1831-1891.

Konev YF, Mitnitskii AB. 1980. Numeric taxonomy of verticillate actino mycetes [dalam bahasa Rusia]. *Mikrobiologiya* (Moscow) 49:110-116.

Korn-Wendisch F, Kutzner HJ. 1992. The Family *Streptomycetaceae*. Di dalam: Balows A, Truper HG, Dworkin M, Harder W, Schleifer KH (ed). *The Prokaryote, A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Application*. Vol. 1, 2nd ed. New York: Springer-Verlag. hlm 921-995.

Krainsky A. 1914. Die Aktinomyceten und ihren Bedeutung in der Natur. *Zentralblatt für Bakteriologie Parasitenkunde infektionskrankheiten und Hygiene. Abteilung II* 41:649-688.

Krassilnikov NA. 1941. *Keys to Actinomycetales*. Translated from Russian 1966 ed. Moscow: Academic of Sciences of the USSR Institute of Microbiology.

Krassilnikov NA, Agre NS, Dorokova LA, Solokov AA. 1968. Three new species of thermophilic actinomycetes [dalam bahasa Rusia]. *Mikrobiologiya* 37:75-83.

Kurylowicz W, Gyllenberg HG. 1988. Problems of taxonomy of streptomycetes. Di dalam: Okami Y, Beppu T, Ogawara H (ed). *Biology of Actinomycetes '88*. Tokyo: Japan Scientific Societies Pr. hlm 11-18.

Kurylowicz W, Paszkiewicz A, Szulga T, Woznicka W, Kurzatkowski W. 1975. Numerical classification and identification methods in industrial microbiology. Di dalam: *Proceedings of the Symposium on Automation in Microbiology*, Copenhagen 1975. hlm 22.

Kurylowicz W, Woznicka W, Gyllenberg HG. 1969. On the taxonomy of *Streptomyces* [dalam bahasa perancis]. *Rev Immunol* (Paris) 33:27-38.

Kurylowicz W, Woznicka W, Paszkiewicz A, Malinowski K. 1970. Application of numeric taxonomy in streptomycetes. Di dalam: Prauser H (ed). *Actinomycetales*. Jena: Veb Gustav Fischer Verlag. hlm 107-122.

Küster E. 1959. Outline of a comparative study of criteria used in characterization of the actinomycetes. *Int Bull Bacteriol Nomenclature Taxon* 9:97-104.

Küster E. 1961. Results of a comparative study of criteria used in the classification of the actinomycetes. *Int Bull Bacteriol Nomenmenclature Taxon* 11:91-98.

Küster E. 1972. Simple working key for the classification and identification of named taxa included in the international *Streptomyces* project. *Int J Syst Bacteriol* 22:139-148.

Langham CD, Williams ST, Sneath PHA, Mortimer AM. 1989. New probability matrices for identification of *Streptomyces*. *J Gen Microbiol* 135:121-133.

Manfio GP. 1995. *Towards Minimal Standards for the Description of Streptomyces species* [Disertasi]. Newcastle upon Tyne: University of Newcastle.

Mayama M. 1959. A consideration on the classification of streptomycetes. *Ann Rep Shionogi Res Lab* (Japan) 9:1185-1212.

Nomi R. 1960. Studies on the classification of streptomycetes Part XIII. The microscopic morphology and other characteristics of whorl forming strains. *J Gen Appl Microbiol* 5:191-199.

Nonomura H. 1974. Key for classification and identification of 458 species of the streptomycetes included in ISP. *J Ferment Technol* 52:78-92.

O'Donnell AG, Falconer C, Goodfellow M, Ward AC, Williams E. 1993. Biosystematics and diversity amongst novel carboxydotrophic actinomycetes. *Antonie van Leeuwenhoek* 64:325-340.

Paszkiewicz A. 1972. Application of numerical methods to the taxonomy of the genus *Streptomyces*. *Archiv Immunol Ther Exp* 20:307-332.

Pridham TG, Hessettine CW, Bennedict RG. 1958. A guide for the classification of *Streptomycetes* according to selected groups. *Appl Microbiol* 6:52-79.

Pridham TG, Tresner HD. 1974a. Family VII. *Streptomycetaceae* Waksman & Henrici 1943, 339AL. Di dalam: Buchanan RE, Gibbons NE (ed). *Bergey's Manual of Determinative Bacteriology*, 8th ed. Baltimore: Williams & Wilkins Co. hlm 747-748.

Pridham TG, Tresner HD. 1974b. Genus I. *Streptomyces* Waksman & Henrici 1 943, 339AL. Di dalam: Buchanan RE, Gibbons NE (ed). *Bergey's Manual of Determinative Bacteriology*, 8th ed. Baltimore: Williams & Wilkins. hlm 747-829.

Saddler GS. 1988. *Selective Isolation and Rapid Identification of Streptomycetes* [Disertasi]. Newcastle upon Tyne: University of Newcastle.

Sahin N. 1995. *Selective Isolation, Characterisation and Classification of Novel Alkaliphilic Streptomycetes* [Disertasi]. Newcastle upon Tyne: University of Newcastle.

Schrempf H, Kessler A, Bronneke V, Dittrich W, Betzler M. 1989. Genetic instability in streptomycetes. Di dalam: Hershberger CL, Queener StW, Hegeman G (ed). *Microbiology '89*. Washington DC: Amer Soc Microbiol. hlm 133-140.

Schrempf H, Muller D, Kampfer P. 1994. Spontaneous variants of strepto-mycetes lack important phenotypic characteristics relevant for numerical identification. Di dalam: *Abstract of the 9th International Symposium on the Biology of Actinomycetes (ISBA'94)*, Moscow, 10-15 Jul 1994. hlm 246.

Sembiring L, Ward AC, Goodfellow M. 2000. Selective isolation and characterisation of members of the *Streptomyces violaceusniger* clade associated with the roots of *Paraserianthes falcataria*. *Antonie van Leeuwehoek* 78:353-366.

Shirling EB, Gottlieb D. 1966. Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 16:313-340.

Shirling EB, Gottlieb D. 1968a. Cooperative description of type cultures *Streptomyces* II. Species description from first study. *Int J Syst Bacteriol* 18:69-189.

Shirling EB, Gottlieb D. 1968b. Cooperative description of type cultures of *Streptomyces* III. Additional species descriptions from first and second studies. *Int J Syst Bacteriol* 18:279-392.

Shirling EB, Gottlieb D. 1969. Cooperative description of type cultures of *Streptomyces* IV. Species Description from the second, the third, and the fourth studies. *Int J Syst Bacteriol* 19:391-512.

Shirling EB, Gottlieb D. 1972. Cooperative description of type cultures of *Streptomyces* V. Additional descriptions. *Int J Syst Bacteriol* 22:265-394.

Silvestri L, Turri M, Hill LR, Gilardi E. 1962. A quantitative approach to the systematics of actinomycetes based on overall similarity. Di dalam: Ainsworth GC, Sneath PHA (ed). *Microbial Classification*. Cambridge: Cambridge Univ Pr. hlm 333-360.

Sneath PHA. 1970. Application of umerical taxonomy to actinomycetales: problems and prospects. Di dalam: Prauser H (ed). *The Actinomycetales*. Jena: Gustav Fischer Verlag. hlm 371-377.

Szabó I, Marton M. 1964. Comments on the first results of the international cooperative work on criteria used in characterisation of streptomycetes. *Int Bull Bacteriol Nomenclature Taxon* 14:17-38.

Szabó I, 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 Bot Acad Sci Hungaricae* 21:387-418.

Szulga T. 1978. A critical evaluation of taxometric procedures applied to *Streptomyces*. *Zentralblaat Bakteriol Parasitenkunde, Infektionskrankenheiten Hygiene Abteilung* 6:31-42.

Trejo WH. 1970. An evaluation of some concepts and criteria used in the speciation of streptomycetes. *Trans New York Acad Sci* Series 2, 32:989-997.

Waksman SA. 1919. Cultural studies of species *Actinomyces*. *Soil Sci* 8:71-215.

Waksman SA. 1957. Family III. *Streptomycetaceae* Waksman & Henrici. Di dalam: Breed RS, Murray EGD, Smith NR (ed). *Bergey's Manual of Determinative Bacteriology* 7th ed. Baltimore: Williams & Wilkins. hlm 744-825.

Waksman SA. 1961. *The Actinomycetes. Classification, identification and descriptions of genera and species*, Volume 2. Baltimore: Williams & Wilkins.

Waksman SA, Curtis RE. 1916. The Actinomyces of the soil. *Soil Sci* 1:99-134.

Waksman SA, Henrici AT. 1943. The nomenclature and classification of the actinomycetes. *J Bacteriol* 46:337-341.

Waksman SA, Henrici AT. 1948. Family *Actinomycetaceae* Buchanan and Family *Streptomycetaceae* Waksman & Henrici. Di dalam: Breed RS, Murray EGD, Hitchens AP (ed). *Bergey's Manual of Determinative Bacteriology* 6th ed., Baltimore: Williams & Wilkins. hlm 929-980.

Waksman SA, Starkey RL. 1924. Influence of organic matter upon the development of fungi, actinomycetes and bacteria in the soil. *Soil Sci* 17:373-378.

Waksman SA, Woodruff HB. 1941. *Actinomyces antibioticus*, a new soil organism antagonistic to pathogenic and non-pathogenic bacteria. *J Bacteriol* 42:231-249.

Williams ST, Davies FL, Hall DM. 1969. A practical approach to the taxonomy of actinomycetes isolated from soil. Di dalam: Sheals JG (ed). *The Soil Ecosystem*. London: Systematics Assoc. hlm 107-117.

Williams ST, Goodfellow M, Alderson G. 1989. Genus *Streptomyces* Waksman and Henrici 1943, 339AL. Di dalam: Williams ST, Sharpe ME, Holt JG, *Bergey's Manual of Systematic Bacteriology* Volume 4. Baltimore: Williams & Wilkins. hlm 2452-2492.

Williams ST, Goodfellow M, Alderson G, Wellington EMH, Sneath PHA, Sackin MJ. 1983a. Numerical Classification of *Streptomyces* and Related Genera. *J Gen Microbiol* 129:1743-1813.

Williams ST, Goodfellow M, Wellington EMH, Vickers JC, Alderson G, Sneath PHA, Sackin MJ, Mortimer AM. 1983b. A probability matrix for identification of some streptomycetes. *J Gen Microbiol* 129:1815-1830.

Williams ST, Wellington EMH, Goodfellow M, Alderson G, Sackin M, Sneath PHA. 1981. The genus *Streptomyces*- a taxonomic enigma. Di dalam: Schaal KP, Pulverer G (ed). *Actinomycetes*, Stuttgart: Gustav Fischer Verlag. hlm 47-57.

Witt D, Stackebrandt E. 1990. Unification of the genera *Streptoverticillium*, *Streptomyces* and amendation of *Streptomyces* Waksman & Henrici 1943, 339AL. *Syst Appl Microbiol* 13:361-371.

Woznicka W. 1965. Taxonomic and taxometric studies on yellow series of actinomycetes [dalam bahasa Polandia]. Warsaw: Panstowowz Zaklad Higieny.