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REVIEW ARTICLE

The taxonomy of *Streptomyces* and related genera

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The streptomycetes, producers of more than half of the 10000 documented bioactive compounds, have offered over 50 years of interest to industry and academia. Despite this, their taxonomy remains somewhat confused and the definition of species is unresolved due to the variety of morphological, cultural, physiological and biochemical characteristics that are observed at both the inter- and the intraspecies level. This review addresses the current status of streptomycete taxonomy, highlighting the value of a polyphasic approach that utilizes genotypic and phenotypic traits for the delimitation of species within the genus.

Keywords: streptomycete taxonomy, phylogeny, numerical taxonomy, fingerprinting, bacterial systematics

Introduction

The genus *Streptomyces* was proposed by Waksman & Henrici (1943) and classified in the family *Streptomycetaceae* on the basis of morphology and subsequently cell wall chemotype. The development of numerical taxonomic systems, which utilized phenotypic traits helped to resolve the intergeneric relationships within the family *Streptomycetaceae* and resulted in the reclassification of six additional genera (*Actinopycnidium*, *Actinosporangium*, *Chainia*, *Elytrosporangium*, *Kitasatoa* and *Microellobosporia*) to the *Streptomyces* genus (Williams *et al*., 1983a; Goodfellow *et al*., 1986a–d). These early numerical systems utilized phenotypic characters, which were fundamentally changed by the incorporation of molecular biological characteristics into classification systems and thus enabled considerable advances for genus delimitation within the *Actinobacteria* (Stackebrandt *et al*., 1997). Prior to this, the genera *Streptomyces* and *Streptoverticillium* were two distinct genera; both have cell-wall type 1 (Lechevalier & Lechevalier, 1970), are lysed by the same phages (Wellington & Williams, 1981) and are phylogenetically closely related (Stackebrandt & Woese, 1981). Immunodiffusion studies (Ridell *et al*., 1986) linked members of the genus *Streptoverticillium* closely to the *Streptomyces lavendulae* species group. Kämpfer *et al*. (1991) also found similarities using physiological tests. Gladek *et al*. (1985) observed differences in DNA–RNA pairing; this and the morphological trait of producing whorls were the only detectable differences between the two genera. Witt & Stackebrandt (1990) concluded from 16S and 23S rRNA comparisons that the genus *Streptoverticillium* should be regarded as a synonym of *Streptomyces*.

Kitasatosporia was also included in the genus *Streptomyces*, despite having differences in cell wall composition, on the basis of 16S rRNA similarities (Wellington *et al*., 1992). This was revoked by Zhang *et al*. (1997), who demonstrated that members of the genus *Kitasatosporia* always formed a stable monophyletic clade away from streptomycetes when sequences from the entire 16S rRNA genes were compared.

Kineosporia and *Sporichthya* are both rare and share many chemotaxonomic similarities with members of the genus *Streptomyces*, which led to their incorporation into the genus (Logan, 1994). The *Kineosporia* and *Sporichthya* have since been reinstated as independent genera on the bases of ribosomal sequencing: *Sporichthya* is a genus of the family *Sporichthyaceae* of the suborder *Frankineae* (Stackebrandt *et al*., 1997) and the *Kineosporia* are grouped with the *Kineococcus* (Kudo *et al*., 1998).

These changes resulted in the genus *Streptomyces* being the sole member of the family *Streptomycetaceae*. In spite of this genus unification, the current classification systems have not yet solved the taxonomy within the genus, a pending issue that will be discussed in this review.

Definitions

Definition of the genus *Streptomyces*

Streptomycetes are Gram-positive aerobic members of the order *Actinomycetales* within the classis *Actinobacteria* (Stackebrandt *et al*., 1997) and have a DNA G+C content of 69–78 mol% (Korn-Wendisch & Kutzner, 1992). Streptomycetes produce an extensive branching substrate and aerial mycelium. The substrate hyphae are approximately 0·5–1·0 μm in diameter and often lack cross-walls during the vegetative phase. Growth occurs at the hyphal apices and is accompanied by branching, thus producing a complex tightly woven matrix of hyphae during the vegetative growth phase. As the colony ages, aerial mycelia (sporophores) are produced which develop into chains of spores (conidia) by the formation of crosswalls in the multinucleate aerial filaments. This is followed by separation of individual cells directly into spores (Wildermuth & Hopwood, 1970). The morphological features of the aerial mycelium are regarded as more significant for characterization than the aerial mycelium and they include the mode of branching, the configuration of the spore chains and the surface of the spores. The surface of the conidial wall often has convoluted projections which, together with the shape and the arrangement of the spore-bearing structures, are characteristic of each species and were often used for the separation of *Streptomyces* species (Pridham *et al*., 1958; Korn-Wendisch & Kutzner, 1992). They can be distinguished from other actinomycetes by their cell wall type which is characterized as Type I *sensu* (Lechevalier & Lechevalier, 1970). The presence of LL-diaminopimelic acid and glycine and the absence of characteristic sugars are typical of this cell wall type. In addition to these traits the acyl type of the muramyl residues in the cell-wall peptidoglycans is acetyl (Uchida & Seino, 1997).

Perspectives regarding the definition of species and overspeciation within the genus

The discovery of antibiotics produced by streptomycetes in the 1940s led to extensive screening for novel bioactive compounds and subsequently the need for patenting led to an overclassification of the genus. Producers of novel natural products were described as new species and patented. Species described within the genus *Streptomyces* increased from approximately 40 to over 3000. Many of these strains were considered to be synonyms. Standard identification criteria and type strains were needed to prevent overspeciation. In 1964, the International *Streptomyces* Project (ISP) was initiated to introduce standard criteria for the determination of species so as to reduce the number of poorly described synonymous species. Shirling & Gottlieb (1968a, b, 1969, 1972) described the standard criteria that involved the use of spore chain morphology, spore surface ornamentation, colour of spores, substrate mycelium and soluble pigments, production of melanin pigment and the utilization of a range of carbon sources. More than 450 *Streptomyces* species were redescribed and type strains were selected and deposited in internationally recognized culture collections. The ISP did not provide an identification scheme, only standard methods by which one could be achieved. Williams *et al*. (1983a) used a numerical taxonomic approach based on phenetic characters which resulted in a reduction of the numbers of described *Streptomyces* species, the 1989 edition of *Bergey's Manual* describes 142 species (Williams *et al*., 1989), in contrast to 463 species described in the 1974 edition (Pridham & Tresner, 1974b). This did not fully resolve the problem of overspeciation, numerous species and subspecies were described and many natural isolates did not match the reference strains used to construct the identification matrices (Goodfellow & Dickenson, 1985). Additional chemotaxonomic and molecular methods are now used together with the numerical taxonomic methods to improve our understanding of species relatedness within the *Streptomyces* genus. These include cell wall composition (Lechevalier & Lechevalier, 1970), phage typing (Wellington & Williams, 1981), DNA–DNA hybridization (Labeda, 1992), ELISA (Kirby & Rybicki, 1986), rapid biochemical assay for utilization of 4-methyl-umbelliferone-linked substrates (Goodfellow *et al*., 1987b), comparison of ribosomal protein patterns (Ochi, 1989), low-frequency restriction fragment analysis (Beyazova & Lechevalier, 1993), and comparisons of 16S rRNA and 23S rRNA sequences (Stackebrandt *et al*., 1991a, b). It is important to note that many of the enumerated methods covered in this review have been applied independently, an integrated study to combine and evaluate a specified standard group of strains is still pending. This is illustrated by Manfio *et al*. (1995) who grouped together data from 10 independent studies encompassing 293 strains from 22 different taxospecies and eight different chemotaxonomic or DNA fingerprinting techniques. Not one taxospecies was represented by all eight tests, and to confound matters the strains studied within the taxospecies were not consistent.

Numerical taxonomy of the streptomycetes

Early taxonomic systems relied on phenotypic traits and streptomycetes were clustered according to observed similarities and differences. Groups were obtained using similarities in spore chain morphology, spore surface ornamentation, colour (spores, substrate mycelium and soluble pigments) and the production of melanin pigment. These groups were further subdivided using carbon source utilization patterns (Pridham & Tresner, 1974a, b). This method has been further developed to produce the presently accepted method for the classification of streptomycetes. Numerical taxonomy was introduced to allow the simultaneous evaluation of a large number of phenotypic traits (Sneath & Johnson, 1972). Williams *et al*. (1983a), conducted a numerical taxonomic study of streptomycetes and related genera with cell wall

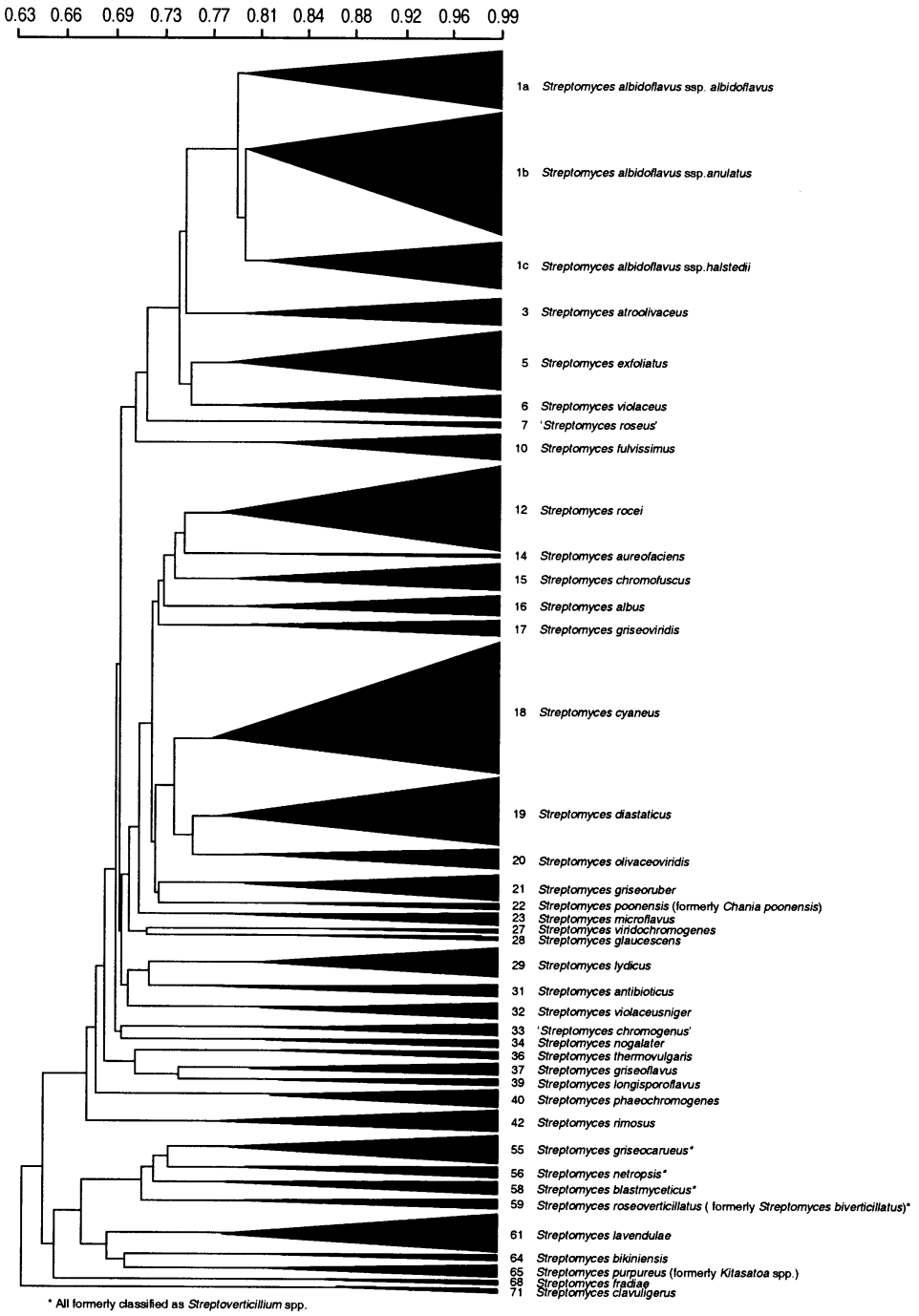

Fig. 1. Simplified dendrogram depicting the taxonomic clusters within the *Streptomyces* genus determined using numerical taxonomy (Williams *et al.*, 1983a).

chemotype I (Lechevalier & Lechevalier, 1970) using phenetic characteristics. A total of 475 strains were characterized using 139 unit characters. The results were analysed with a standard resemblance coefficient, the average linkage algorithm. Williams *et al.* (1983a) also subdivided the genus *Streptomyces* into species groups. *Streptomyces* type strains (394) were clustered according to similarities obtained from the phenetic tests. At the 77·5% S_{SM} level, 19 major, 40 minor and 18 single strain clusters were recovered. Many of the minor clusters consisted of less than five strains. These were regarded as species: for example, cluster 28 contained '*Streptomyces flavoviridis*' ISP 5153 and '*Streptomyces glaucescens*' ISP 5155; both are considered to be *Streptomyces glaucescens*. Major clusters varied in size from six to 71 strains. Each cluster could be regarded as single species despite the high diversity observed within some clusters and these therefore were

regarded as species-groups. The largest species-group is *Streptomyces albidoflavus* (cluster 1) and contains 71 type strains, including 44 type strains, 15 invalidly published species and 12 unnamed strains (Fig. 1). This cluster is further subdivided into three: cluster 1a, *Streptomyces albidoflavus* subsp. *albidoflavus* (20 strains); cluster 1b, *Streptomyces albidoflavus* subsp. *anulatus* (38 strains) and cluster 1c, *Streptomyces albidoflavus* subsp. *halstedii* (13 strains) (Williams *et al*., 1989). Their phenetic characteristics included yellow/grey pigmentation, no melanin production, smooth spores which grow in straight chains and resistance to a number of antibiotics including penicillin, lincomycin and cephaloridine. Many of the strains were patented antibiotic producers. Overall the group does show general antimicrobial activity; 39% produce compounds with antifungal activity, 32% produce compounds with anti-Gram-positive activity and 10% are active against Gram-negative micro-organisms (Williams *et al*., 1983b). This cluster clearly exemplifies the problems with streptomycete systematics.

A probabilistic database for the identification of streptomycetes was constructed using information from the numerical taxonomic database (Williams *et al*., 1983b). The identification matrix used 41 characteristics to diagnose 23 groups using MATIDEN (Sneath, 1979). This program calculated the Willcox coefficient (Willcox *et al*., 1973). A Willcox score of 0·85 was taken as being sufficient to allow for a positive identification. The MATIDEN program also calculates the taxonomic distance (and its standard error) of the isolates to the nearest other two clusters. A similar matrix devised by Goodfellow *et al*. (1987a) to classify thermophilic streptomycetes using numerical taxonomy was able to cluster streptomycetes that could grow at 55 °C into eight clusters.

The identification matrix of Williams *et al*. (1983b) was updated by Langham *et al*. (1989) to improve the resolution within the subclusters by including minor clusters as well as major clusters. The minor clusters were defined using either one or two strains, making it less valid statistically than the identification matrix of Williams *et al*. (1983b). Different tests were also used to define the major clusters, including an increased number of antibiotic resistances. Huddleston *et al*. (1997) demonstrated the variability of antibiotic resistance within taxonomic groups defined using the Williams *et al*. (1983b) identification matrix.

Kämpfer *et al*. (1991) revised the numerical classification system of Williams *et al*. (1983a) using 329 tests and 821 strains, including many that were not included in the Williams study. The identification matrix of Kämpfer *et al*. (1991) compared the numerical phenetic data that they obtained with published genetic and chemotaxonomic data. The major phena detected by Williams *et al*. (1983a) were maintained, however cluster-groups were only detected in part.

Identification systems based on phenotypic characters (chemotaxonomy)

Chemotaxonomic methods have long been used to distinguish streptomycetes from other actinomycetes (Lechevalier & Lechevalier, 1970). The development of reliable, rapid and sensitive analytical methods such as electrophoresis, chromatography and spectroscopy has led to the development of alternative chemotaxonomic approaches for streptomycete classification (Goodfellow & O'Donnell, 1993).

Fatty acids

Members of the genus *Streptomyces* have straight chain, iso- and anteiso-branched chain fatty acids with a carbon chain-length of 14–18 atoms (Hofheinz & Grisebach, 1965; Lechevalier, 1977; Popisil *et al*., 1985; Saddler *et al*., 1986, 1987). Some species also have hydroxylated methyl esters (Kroppenstedt, 1990). Fatty acid analysis has been used to distinguish between bacterial genera (Minnikin & O'Donnell, 1984; Goodfellow, 1989; Kaneda, 1991). It has also been used in the delimitation of species groups in *Bacterioides* (Brondz *et al*., 1991) and strain determination in *Corynebacterium* (Gudmestad *et al*., 1988). For fatty acid analysis to be of use below the genus level, standardized growth and analytical conditions are required to ensure reproducible fatty acid profiles that are suitable for multivariate statistical analysis (Saddler *et al*., 1986). Fatty acid profiles were used to investigate the taxonomy of strains of *Streptomyces cyaneus* and soil isolates which shared the blue spore trait (Saddler *et al*., 1987). Fatty acid methyl esters (FAMEs) were characterized using gas chromatography and the data were analysed using the SIMCA statistical package. The streptomycetes formed three cluster groups; the first cluster contained the majority of the *Streptomyces cyaneus* strains, the second contained the rest of the *Streptomyces cyaneus* strains and members of other streptomycete species groups and the final cluster contained the blue-spored soil streptomycetes. This study demonstrated that fatty acid analysis could be used to assign unknown streptomycetes to established taxa, and that the *Streptomyces cyaneus* taxon was heterogeneous. In contrast, FAME analysis was unable to delimit 11 *Streptomyces violaceoniger* strains from 74 type strains and natural isolates in a similar study (Phillips, 1992). In spite of the non-reliability of the fatty acid composition for species identification within the genus *Streptomyces*, FAME patterns obtained using standardized conditions are still of high value for the rapid characterization of large numbers of wild-type streptomycetes isolated from the environment, independently from the taxonomic status of each isolate (Saddler *et al*., 1987).

Whole-cell analysis

Curie-point pyrolysis mass spectrometry (PyMS) has been applied to the classification, identification and

typing of actinomycetes (Sanglier *et al*., 1992). Whole cells are subject to high temperature, non-oxidative thermal degradation and the resulting pyrolysate is analysed using mass spectrometry, providing a fingerprint for the organism that can be used quantitatively to discriminate between strains. As with fatty acid analysis, stringent standardization is required to ensure reproducibility. Changes in growth media and incubation time prior to pyrolysis affect the pyrogram obtained. Sanglier *et al*. (1992) used PyMS to analyse strains from the largest *Streptomyces* species group, *Streptomyces albidoflavus*, and were able to divide the *Streptomyces albidoflavus* and *Streptomyces anulatus* strains into distinct groups. Three of the six *Streptomyces halstedii* strains investigated also clustered into a distinct group, whereas the remaining strains clustered within the other two groups. This is supported by the studies of Kämpfer *et al*. (1991), which recognized that *Streptomyces albidoflavus* strains and *Streptomyces anulatus* strains are separate genomic species. Interestingly, they also found that *Streptomyces anulatus* ISP 5361^T, the strain used to name the *Streptomyces anulatus* cluster, formed an independent cluster, so in this report the *Streptomyces anulatus* cluster was renamed *Streptomyces griseus*.

Biochemical tests in streptomycete taxonomy

Goodfellow *et al*. (1987b) investigated the use of rapid biochemical tests for streptomycete taxonomy by using a fluorogenic probe which was conjugated to the substrate and fluoresced after hydrolysis by the target enzyme. In this study streptomycete strains from 17 taxonomic clusters as defined by Williams *et al*. (1983a) were investigated for 14 different enzyme activities. Reproducible results were obtained; however, a discontinuous distribution of enzymes was detected in all of the test strains, which raises questions as to the usefulness of this method as a taxonomic tool for the genus *Streptomyces*.

Serology

Ridell *et al*. (1986) raised antisera against the mycelia from streptomycetes, streptoverticillia and *Nocardiopsis* sp. The close relationship between streptomycetes from cluster 61 (*Streptomyces lavendulae*) and the streptoverticillia was confirmed. There was also cross-reactivity between one of the two *Nocardiopsis dassonvillei* strains tested with antibodies raised against '*Streptoverticillium flavopersicum*' and *Streptomyces griseus*. Kirby & Rybick (1986) also investigated one use of antisera for streptomycete taxonomy by raising antisera against *Streptomyces griseus* (*Streptomyces anulatus*, cluster 1B of Williams *et al*., 1983a) and '*Streptomyces cattleya*' (cluster 47). The resulting antisera were genus-specific and showed some degree of species-specificity too. Though these studies were successful in demonstrating the use of antisera for defining cluster groups, this approach does not have the reproducibility and specificity of monoclonal antibodies. Wipat *et al*. (1994) raised monoclonal antibodies against '*Streptomyces lividans*' 1326 spores, and antibodies from the hybridoma cell lines obtained were screened for specificity to '*Streptomyces lividans*' 1326. Antibodies were detected that were specific for the cluster group containing '*Streptomyces lividans*' (cluster 21, Williams *et al*., 1983a), thus supporting the grouping put forward by Williams *et al*. (1983a). One limitation to using monoclonal antibodies to classify soil streptomycetes is that the low-nutrient environment may cause changes in the accessibility and expression of surface antigens, which might affect antibody binding. This has been demonstrated for Gram-negative micro-organisms (Nelson *et al*., 1991) and there is limited evidence that it may also occur with Gram-positive micro-organisms (Smith *et al*., 1991).

Phage typing

Actinophages can be used for host-identification at the genus and the species level (Wellington & Williams, 1981). Streptomycete phages can be either polyvalent, such as ØC31 (Chater *et al*., 1986), or species-specific, some of which are listed in Table 1. Polyvalent streptomycete phage can infect a wide number of members within the genus; Klaus *et al*. (1981) screened the *Streptomyces hygroscopicus* phage SH10 against 36 strains from different species groups. The 28 strains that were susceptible represented 18 different species groups. In general, streptomycete phages are genus-specific (Korn-Wendisch & Schneider, 1992), although some cross-reactivity has also been detected with other genera, including *Nocardia*, *Streptosporangium* and *Mycobacteria* (Bradley *et al*., 1961). Species-specific actinophage do not infect all the members of the host species group. For example, in host-range studies by Korn-Wendisch & Schneider (1992), three *Streptomyces coelicolor* Müller phages (designated 14, 24 and 233) were screened against 67 members of the host species group. Most of the streptomycetes tested were susceptible to the three phage (61/67, 58/67 and 60/67, respectively). In the same study, only 22 of 40 *Streptomyces albus* strains were susceptible to the *Streptomyces albus* species-specific phage S3. This low number may be due either to misclassification of strains or to host phage resistance mechanisms (reviewed by Chater *et al*., 1986). Resistance can be mediated by restriction modification systems (Cox & Baltz, 1984), the integration of a similar phage, or adsorption specificity (Lomovskaya *et al*., 1982). The use of phage for streptomyces taxonomy is often overlooked, and they are often avoided in industrial situations to prevent problems that may occur with phage in fermentation plants. An example of this is phi TG1, which was isolated from the thienamycin producer *Streptomyces catteleya* (Foor *et al*., 1985). Disabled phage that are lysogenic and unable to cause host-cell lysis are useful for the construction of integrative vectors, which can be used for the genetic engineering of antibiotic biosynthetic pathways (Foor

Table 1. Species-specific actinophages of the genus *Streptomyces*

Phage	Host	Host species group	Host cluster no.	Reference
98	*S. coelicolor* Müller ATCC 23899^T	*S. albidoflavus*	1A	Wellington & Williams (1981)
14, 24, 233	*S. coelicolor* Müller ATCC 23899^T	*S. albidoflavus*	1A	Korn-Wendisch & Schneider (1992)
89, DP9	*S. griseus* ATCC 23345^T	*S. albidoflavus*	1B	Wellington & Williams (1981)
90	*S. griseinus* ATCC 23915^T	*S. albidoflavus*	1B	Wellington & Williams (1981)
33	'*S. scabies*' ATCC 23962	*S. atroolivaceus*	3	Wellington & Williams (1981)
SV1, SV2	*S. venezuelae* ATCC 10712^T	*S. violaceus*	6	Stuttard (1982)
41	*S. matensis* ATCC 23935^T	*S. rochei*	12	Wellington & Williams (1981)
S3	*S. albus* DSM 40313^T	*S. albus*	16	Korn-Wendisch & Schneider (1992)
SAt1	*S. azureus* ATCC 14921^T	*S. cyaneus*	18	Ogata *et al.* (1985)
100	'*S. caesius*' ATCC 19828	*S. griseoruber*	21	Wellington & Williams (1981)
4, 5a, 5b, 49	*S. violaceoruber* DSM 40049^T	*S. violaceoruber*	SMC*	Korn-Wendisch & Schneider (1992)

* SMC, Single-member cluster (Williams *et al.*, 1989).

et al., 1990). Phage typing of streptomycetes is therefore also useful for determining the host range of these phage based vectors.

Protein profiling

Bacterial whole-cell protein profiles can be analysed by polyacrylamide gel electrophoresis (PAGE) to generate complex banding patterns that can be used to delimit species and subspecies groups. Proteins can be analysed either by using one-dimensional (1-D) or two-dimensional (2-D) protein electrophoresis. 1-D protein electrophoresis allows many bacterial strains to be compared rapidly and reproducibly (Goodfellow & O'Donnell, 1993). Manchester *et al.* (1990) investigated the use of SDS-PAGE to analyse and compare the cellular protein profiles of 32 *Streptomyces* strains and five *Streptoverticillium* strains. Taxonomic correlations were found between the profiles obtained and the phenotypic groupings observed by Williams *et al.* (1983a). Correlations between protein profiles and DNA hybridization groupings have also been demonstrated. Paradis *et al.* (1994) used PAGE and DNA–DNA hybridizations to elucidate the taxonomy of streptomycete isolates that are the causal agent of common potato scab. Isolates obtained from potato tubers were divided into two groups with a correlation coefficient of 0·75 using SDS-PAGE analysis. The same two groups were resolved at approximately 44% similarity using DNA–DNA hybridization analysis. The protein profiles also enabled further differentiation within the two groups; one group contained two subgroups which clustered at a level of 0·82. In the same study, fatty acid analysis results did not correlate with the SDS-PAGE and the DNA–DNA hybridization groupings. This is partly due to the influence of growth conditions on the profiles obtained (Saddler *et al.*, 1986, 1987). Despite the success of these tests to taxonomically group the *Streptomyces scabies* strains, they could not differentiate between pathogenic and non-pathogenic strains.

Multilocus enzyme electrophoresis (MLEE) is another term for the characterization of actinomycetes according to the relative mobilities of cellular enzymes in a gel matrix, and it is used to generate statistical estimates of genetic diversity (Girgis & Schwencke, 1993; Wasem *et al.*, 1991). Oh *et al.* (1996) demonstrated how MLEE could be used for both inter- and intraspecific characterization of streptomycetes, providing the appropriate enzymes were used.

2-D PAGE of total cellular proteins gives a greater resolution of individual gene products than 1-D PAGE (O'Farrell, 1975). This approach is therefore too sensitive to investigate proteins with high evolutionary rates, although it is useful for the analysis of bacterial ribosomal proteins which, due to structural and functional constraints, have a lower evolutionary rate than other proteins (Hori & Osawa, 1987). Mikulik *et al.* (1982) first investigated the variability of ribosomal proteins for use in streptomycete taxonomy, and Ochi (1989) applied 2-D PAGE to investigate the variability of the ribosomal proteins within the streptomycetes. This was further investigated by focusing on AT-L30 proteins, which have genus-specific 2-D PAGE profiles and can be used for the rapid classification and identification of streptomycetes (Ochi, 1992). Ochi (1995) sequenced the N termini from the ribosomal AT-L30 protein of 81 streptomycetes from different taxonomic groups to investigate the phylogenetic groupings within the genus.

Taxonomic studies of *Streptomyces* can also be done by isolating specific proteins from cell extracts and determining the protein sequence so that divergence between strains can be evaluated. Taguchi *et al.* (1996) used the *Streptomyces* subtilisin inhibitor protein (SSI), which plays unidentified role(s) in physiological and/or morphological regulation, to investigate the taxonomic status of the *Streptomyces coelicolor* strains. The SSI-like proteins have molecular masses of 9–12 kDa and show significant amino acid sequence homology in the conserved regions that are required for maintaining the tertiary structure of the SSI. The

amino acid sequence of SSI from '*Streptomyces lividans*' 66, *Streptomyces coelicolor* Müller ISP5233^T and '*Streptomyces coelicolor*' A3(2) were compared. The alignments supported ribosomal sequence comparisons, indicating that '*Streptomyces coelicolor*' A(3) is more closely related to '*Streptomyces lividans*' 66 (cluster 21) than to the type strain, *Streptomyces coelicolor* Müller ISP5233^T (cluster 1).

Genotypic approaches to determining relatedness

The application of molecular techniques to the analysis of bacterial genomes has contributed considerably to our knowledge of bacterial taxonomy. In addition to clustering organisms taxonomically, some of these methods, such as the sequencing of specific genes have also provided an insight into the phylogenetic relationships of the prokaryotes at the genus, species and subspecies level. The taxonomic values of different molecular techniques are listed in Table 2 and specific examples for each, as applied to streptomycetes, are discussed in this section.

DNA–DNA hybridizations

DNA–DNA hybridizations of total chromosomal DNA have been used to determine species identity within the streptomycetes. This is performed by monitoring the reassociation of single-stranded DNAs from different organisms. The degree of relatedness is expressed as % homology and the genomic definition of species is considered to encompass strains with $\geqslant 70\%$ DNA–DNA relatedness and $\leqslant 5\,^{\circ}C$ difference in the melting temperature (ΔT_m) between the homologous and heterologous hybrids formed using standard stepwise denaturization conditions (Wayne *et al*., 1987). Mordarski *et al*. (1986) compared the numerical and phenetic groupings of strains of the *Streptomyces albidoflavus* cluster 1 with DNA–DNA hybridization. As in previous numerical studies, the *Streptomyces albidoflavus* cluster could be subdivided into three. *Streptomyces albidoflavus* subcluster *albidoflavus* was distinct. The two additional subclusters did not resemble previous groupings of *anulatus* or *halstedii*. DNA–DNA hybridizations can also be used to classify streptomycetes at the genus level. Witt & Stackebrandt (1990) used this method to unify the genus *Streptoverticillium* with the streptomycetes. DNA–DNA hybridization values ranged from 15 (*Streptomyces coelicolor* Müller ISP5233^T and *Streptomyces rimosus* ISP 5260^T) to 97% (*Streptomyces lavendulae* ISP 5069^T and '*Streptomyces colombiensis*' ISP 5558) with members of the *Streptoverticillum* genus having cross-hybridization values of between 17 and 25% with streptomycetes. Labeda and co-workers have investigated the use of DNA relatedness to determine coherence of the major streptomycete phenetic groups. In studies using the verticil-forming streptomycetes (formerly *Streptoverticillium* species) (Labeda, 1996), *Streptomyces cyaneus* (Labeda & Lyons, 1991a), *Streptomyces lavendulae* (Labeda, 1993) and *Streptomyces violaceusniger* (Labeda & Lyons, 1991b), the degree of DNA relatedness was discrepant with phenetic groupings. The DNA–DNA hybridization technique is not suitable for use as a single taxonomic test for determining interrelationships between species and species groups, especially where there is considerable genetic instability of certain regions within the chromosome (Redenbach *et al*., 1993).

Restriction digests of total chromosomal DNA

Low-frequency restriction fragment analysis (LFRFA) is another molecular technique that uses the entire bacterial chromosome to provide taxonomic information. The principle of the method is to digest total chromosomal DNA with restriction endonucleases that cut infrequently. In the case of streptomycetes, rare AT cutters are used. The resulting fragments are examined by pulsed-field gel electrophoresis (PFGE) to provide a restriction fingerprint that is indicative of relatedness. Beyazova & Lechevalier (1993) investigated 59 strains from eight species groups within the *Streptomyces* genus by this method and were able to demonstrate its utility for the clustering of some strains. There were however some discrepancies, for example *Streptomyces cyaneus* strains cluster at 90% similarity using physical tests, in contrast to LFRFA results where the same strains group at 58% similarity. Therefore, PFGE/LFRFA reflects the heterogeneity of the *Streptomyces cyaneus* cluster and is useful for finding very closely related

Table 2. Specificity of molecular techniques as applied to streptomycete taxonomy (adapted in part from Ludwig & Schleifer, 1994, and Vandamme *et al.*, 1996)

Target	Method	Specificity
Total chromosomal DNA	DNA–DNA hybridization	Genus to species
	Restriction with rare restriction endonucleases (RFLP, LFRFA)	Species to strain
Randomly cloned or amplified DNA fragment	Isolation and cloning of non-cross-hybridizing fragments	Species to strain
Protein-coding gene or gene fragment	Isolation and sequencing of genes or fragment	Species to strain
16S/23S rRNA	Comparative sequence analysis	Family, genus, species

strains, but it cannot resolve interspecific relationships; PFGE/LFRFA can also be misleading if there are large chromosomal amplifications or deletions (Rauland *et al*., 1995).

Randomly amplified polymorphic DNA (RAPD) PCR assays

RAPD-PCR assays (Williams *et al*., 1990) use single primers with arbitrary nucleotide sequences to amplify DNA, using a low annealing temperature so that polymorphisms can be detected. The resulting pattern of PCR products provides a characteristic fingerprint that enables chromosomal differences between isolates to be detected without having any prior knowledge of the chromosomal sequence.

RAPD-PCR is a rapid method by which streptomycetes can be screened for strain similarity. It does, however, require stringent standardization of the reaction parameters. These include primer sequence, annealing temperatures, buffer components, concentration and quality of template DNA. The arbitrary primer sequence used can affect the usefulness of this technique; Mehling *et al*. (1995) failed to detect any characteristic banding patterns for closely related species unless a highly specific actinomycete primer was used. The resulting fingerprints contained a limited number of bands (one to four), reducing the effectiveness of the method as a taxonomic tool for species delimitation. A similar observation was made by Huddleston *et al*. (1995) who evaluated the use of RAPD-PCR for the resolution of interspecific relationships among members of the *Streptomyces albidoflavus* cluster. These studies also exemplified the variability of the profiles obtained with changes to the concentration of the target DNA (Huddleston *et al*., 1995) and the reaction mixture (Mehling *et al*., 1995). Mehling *et al*. (1995) only obtained reproducible results when Vent DNA polymerase was used instead of *Taq* DNA polymerase. There are several possible explanations as to why this was observed: both enzymes operate at different reaction temperatures, in different buffers and have different proofreading capabilities. With optimization this method can be used at the *Streptomyces* species level. Anzai *et al*. (1994) demonstrated the variation in fingerprints obtained when a single base was substituted on the arbitrary primer; 11 primers were investigated and banding patterns ranged from zero to 20, the most significant differences were observed when the sequence at the 3′ end was altered. In the same study, Anzai *et al*. (1994) investigated the relationship of *Streptomyces virginiae* strains to *Streptomyces lavendulae* strains by RAPD-PCR. It had previously been proposed that *Streptomyces virginiae* was a synonym of *Streptomyces lavendulae* (Williams *et al*., 1983a). RAPD-PCR results were compared with the results for DNA–DNA hybridization, LFRFA, cultural and physiological tests. Consistent results were obtained using all these methods after RAPD-PCR optimization. However, the interspecific relationship of *Streptomyces lavendulae* and *Streptomyces virginiae* could not be sufficiently resolved, though it was possible to distinguish between duplicate strains.

Nucleic acid sequence comparisons

Target genes

The comparison of rRNA sequences is a particularly powerful tool in streptomycete taxonomy. rRNA sequence comparisons have also been useful for answering questions concerning the horizontal transfer of genes within the genus (Huddleston *et al*., 1997). These genes are highly conserved within bacteria. Three regions within the 16S rRNA gene have been observed to have enough sequence variation so as to be useful for genus-specific (α and β regions) and species-specific (γ regions) probes (Fig. 2) within the *Streptomyces* genus (Stackebrandt *et al*., 1991a, b, 1992). 23S rRNA, 5S rRNA genes (Mehling *et al*., 1995) and ribosomal protein sequences have also been used to investigate species relationships within the genus *Streptomyces* (Liao & Dennis, 1994; Ochi, 1995).

rRNA sequences used alone can be misleading due to intraspecific variation and intragenomic heterogeneity. Clayton *et al*. (1995) found a high degree of intraspecific variation within the prokaryotic sequence database. Additional genes have been used to examine inter- and intraspecific relationships in *Frankia* (Hirsh *et al*., 1995) and rhizobia (Young, 1996). Genes that are conserved between species, such as housekeeping genes (e.g. elongation factors and ATPase subunits), are the primary target genes to be studied (Ludwig & Schleifer, 1994). Huddleston *et al*. (1997) used tryptophan synthase genes in addition to 16S rRNA comparisons to determine the phylogeny of streptomycin-producing streptomycetes and provide evidence for the horizontal transfer of antibiotic resistance genes.

Sequence analysis

Sequence analysis of rRNA genes has been applied to streptomycete taxonomy to investigate relationships at the genus, species and strain level. The use of 16S rRNA is reviewed by Stackebrandt *et al*. (1992), highlighting the importance of the region selected for comparison. Relationships obtained differed according to the variable region (α, β and γ) that was studied. Kataoka *et al*. (1997) conducted a comprehensive study of the γ region from 89 streptomycete type strains that represented several of the major clusters as defined by Williams *et al*. (1983a). The study verified that these variable regions can be used to resolve inter- and intraspecies relationships within the streptomycetes, despite being too variable for determining generic relationships. Of the 89 strains investigated, 57 variants were detected and 42 strains had unique sequences. Since that publication, the same group has subsequently sequenced the γ regions from 485 streptomycete strains and deposited them in GenBank. This is the largest publically available set of

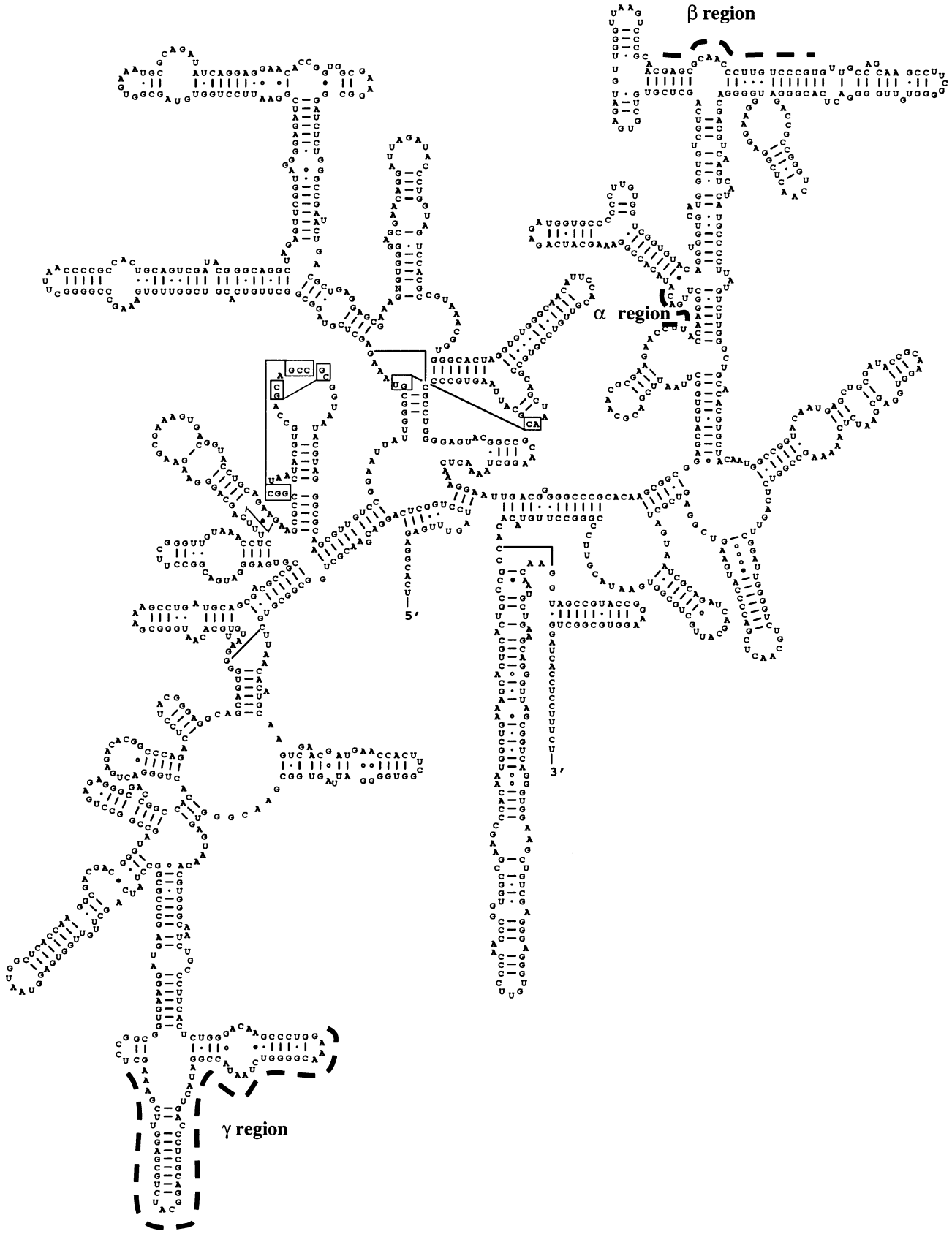

Fig. 2. Secondary structure of 16S rRNA from *Streptomyces coelicolor*. The variable regions that are considered to be diagnostic for the *Streptomyces* genus are marked (*α*, *β* and *γ*) and correspond to nucleotides 982–998, 1102–1122 and 158–203, respectively. Adapted from a figure kindly provided by F. A. Rainey.

(a)

Cluster #	Type species	γ 16S rDNA sequence
1A	*S. albidoflavus*	TCTAATACCGGATA.TGACTGTCCATCGCATGGT.GGATGGTGTAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGG
19	*S. diastaticus*	TCTAATACCGGATA.TGACCGTCCATCGCATGGT.GGATGGTGTAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGG
6	*S. violaceus*	TCTAATACCGGATATTGACCACGCAGGCATGTG.TGGAGGTTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGG
68	*S. fradiae*	TCTAATACCGGATAC.GACCACGTTCAGGCATCTGATGGAGGTTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGGTAAGGCTCACCAAGGCGACGACGGG
17	*S. griseoviridis*	TCTAATACCGGATACTGATCGCGCATGGGCATCCA.GCGGGTTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGGTGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGG
18	*S. cyaneus*	TCTAATACCGGATCCTGACGATCTTGCGGCATCCA.ACGGGTTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGG
12	*S. rochei*	TCTAATACCGGATA.TGACGATCTTGCGGCATCCT.GGATGGTGTAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGG
15	*S. chromofuscus*	TCTAATACCGGATA.CGAGCCGCTCGCATGGT.GGGGGTTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAAGGCTCACCAAGGCGACGACGGG
10	*S. fulvissimus*	TCTAATACCGGATAACGACGCTCTCGCATGGG.AGGGGGTTGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGG
37	*S. griseoflavus*	TCTAATACCGGATAATGACTTCTCCTCGCATGGG.GAGGGGTTGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGG
20	*S. olivaceoviridis*	TCTAATACCGGATACCGACGCTGCAGGCATCTG.TGAGGGTTGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGG
21	*S. griseoruber*	TCTAATACCGGATATCGACGCTGCAGGCATCTG.TGAGGGTTGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGG
40	*S. phaeochromogenes*	TCTAATACCGGATAACGACGCTGCGAGGCATCTC.GGTGGGTTGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGAAGCTCACCAAGGCGACGACGGG
58	*S. blastmyceticus*	TCTAATACCGGATAATGACGTGCGAGGCATGTC.GGTGGGTTGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGG
1B	*S. anulatus*	TCTAATACCGGATAACGACGCTGTCCGCGCATGGG.ACGGGGTTGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGG
1C	*S. halstedii*	TCTAATACCGGATAACGACGCTGTCCCGCATGGG.ACGGGGTTGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGG
3	*S. atroolivaceus*	TCTAATACCGGATAACGACGCTGTCCGCATGGG.GGGGGGTTGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGG
5	*S. exfoliatus*	TCTAATACCGGATAACGACGGCTTCCGCATGGG.AGCGGGTTGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGG
53	*A. mediterranei*	TCTAATACCGGATATCGACAAGCTCTCGCATGGG.GGGTTGTTGAAAGTTCTGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGG
61	*S. lavendulae*	TCTAATACCGGATACCGACGCCTGCCCGCATGGG.GGGGGTTGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCCCACCAAGGCGACGACGGG
16	*S. albus*	TCTAATACCGGATATGGACACGGGATCGCATGGTCTCGGTGTTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGG
29	*S. lydicus*	TCTAATACCGGATACGGACACGGGGTCGCATGACCTCCGGTGTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGG
42	*S. rimosus*	TCTAATACCGGATATGGACACGGGACCGCATGGTCTGGTGTTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGG
32	*S. violaceusniger*	TCTAATACCGGATATGGACTGCCGACCGCATGGTCTGGTGTTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGG
55	*S. griseocarneus*	TCTAATACCGGATACGGACCGCTGACGCATGGT.TGGTGGTTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGG
consensus		**TCTAATACCGGATa Ac c c cgCATgg gggggT gAAAGcTCcGGCGGTGcAGGATGAgCCCGCgGCCTATCAGCTtGTTGGTGaGGTaatgGCtcACCAAGGCGACGACGGG**

(b)

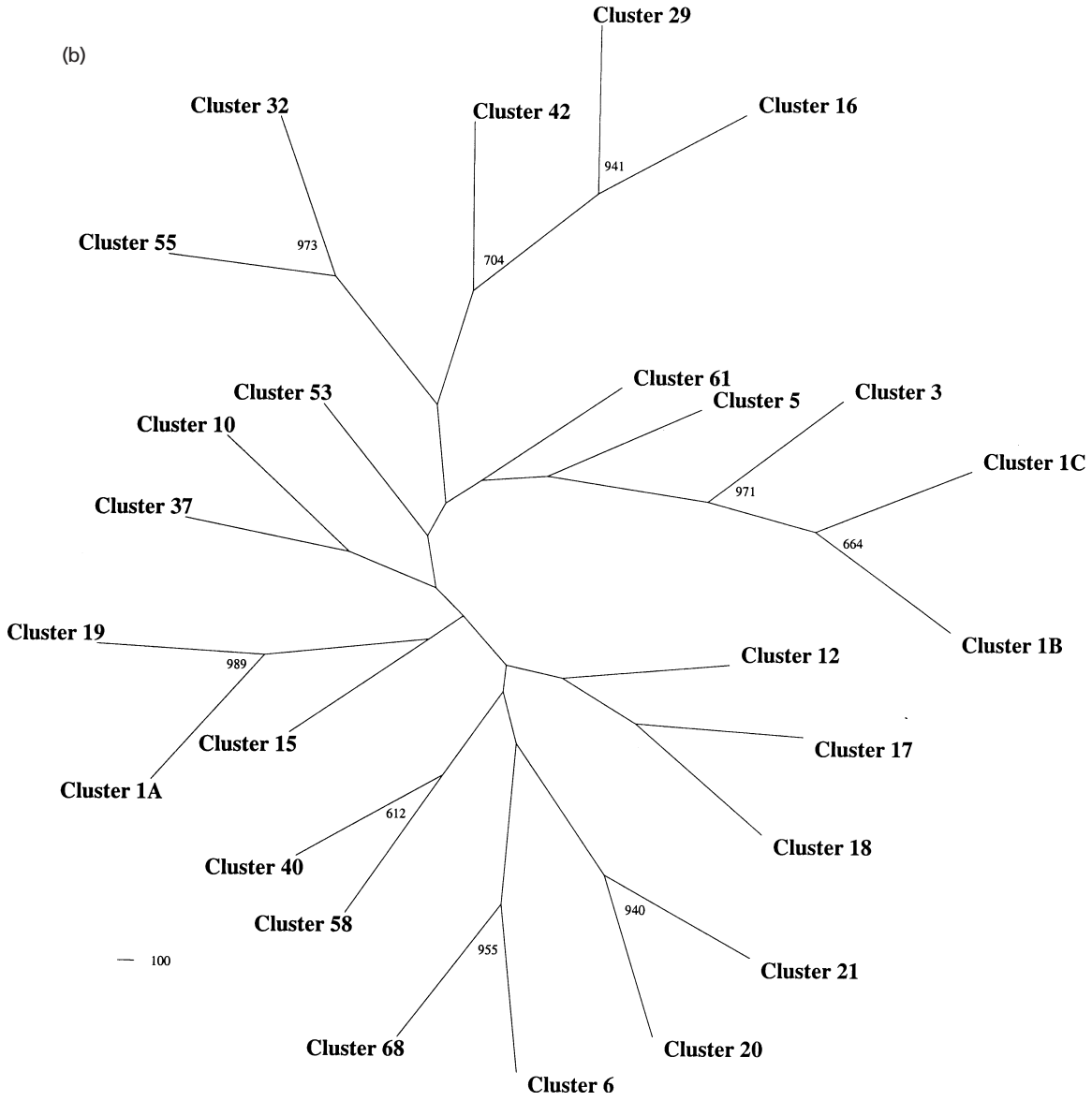

Fig. 3. Genotypic analysis of the major streptomycete clusters, as defined by Williams *et al*. (1983a). Comparison of the 16S rDNA from nucleotides 158–276 including the γ region (variable regions are shaded) (a), and phylogenetic

streptomycete 16S rDNA sequence data. Comparison of the γ regions from representatives of the major cluster groups defined by Williams *et al.* (1983a) supports the taxonomic status of the phenotypic groups (Fig. 3a); the only strains with identical γ regions were *Streptomyces olivaceoviridis* and *Streptomyces griseoruber* representatives from clusters 20 and 21, respectively. Analysis of the *Streptomyces albidoflavus* group, which had previously been shown to comprise three species groups and over 60 strains (Williams *et al.*, 1989), was resolved into six groups using sequence comparisons of the γ region (Kataoka *et al.*, 1997). Interestingly, the three phenotypic subgroups were maintained, although they did not cluster together; this is also the case when members of the major cluster groups as defined by Williams *et al.* (1983a) are analysed phylogenetically (Fig. 3b).

Hain *et al.* (1997) also investigated the use of 16S rRNA probes to determine intraspecific relationships within *Streptomyces albidoflavus* and found sequence comparison to be useful for species delimitation, but of no value for strain differentiation. The intergenic 16S–23S rRNA spacer regions were more useful for investigating the intraspecific relationships of the *Streptomyces albidoflavus* cluster. *Streptomyces* genus-specific probes have also been developed for the 23S rRNA gene (Mehling *et al.*, 1995) and the 5S rRNA gene (Park *et al.*, 1991). 5S rRNA sequence comparisons of acidophilic and neutrophilic streptomycetes confirmed the taxonomic status of *Chainia*, *Elytrosporangium*, *Kitasatoa*, *Microellobosporia* and *Streptoverticillia* spp. as members of the genus *Streptomyces* (Park *et al.*, 1991). 16S rRNA comparisons have also been used to investigate the taxonomic status of natural isolates. In a study by C. Kemmerling and others (personal communication), the 16S rRNAs of 14 marine streptomycetes were compared. While some of these isolates could be assigned to known sequence groups, the majority could not. When studying natural isolates the sequence database is still too incomplete for taxonomic studies. There are 464 validly described *Streptomyces* species and 45 subspecies (Hain *et al.*, 1997). RNA sequence data were presented for approximately 120 *Streptomyces* strains that encompass approximately 70 species groups (Embley & Stackebrandt, 1994). However, considerably more sequence data are now publically available for defining phylogenetic relationships within and between species (Maidak *et al.*, 2000). Problems in identifying new strains to existing species persist and underline the need for a polyphasic approach to streptomycete taxonomy at the species level, using a combination of phenotypic, genotypic and phylogenetic tests as originally outlined for bacterial taxonomy by Colwell (1970).

Sequence comparisons of specific genes have also been applied to investigate the relationship of specific groups of streptomycetes such as antibiotic producers (Huddleston *et al.*, 1997) and pathogenic streptomycetes (Takeuchi *et al.*, 1996; Bramwell *et al.*, 1998). Lambert & Loria (1989a, b) used phenotypic traits to characterize streptomycetes that cause common potato scab disease. They concluded that the most virulent pathogens could be classified into two species: *Streptomyces scabiei* and *Streptomyces acidiscabiei*. *Streptomyces acidiscabiei* is pathogenic in soils with a pH below 5·2. Takeuchi *et al.* (1996) used phylogenetic analysis by sequencing the complete 16S rRNA to investigate virulent strains isolated from diverse habitats and demonstrated that rather than the virulent strains falling into two groups, they were taxonomically diverse. This was also confirmed in a separate study by Bramwell *et al.* (1998). Huddleston *et al.* (1995, 1997) used a polyphasic approach to investigate the taxonomic status of streptomycin-resistant type strains and natural isolates. Numerical taxonomy was performed alongside LFRFA, comparisons of 16S rRNA and the housekeeping gene that encodes tryptophan synthetase (*trpA/B*). There was no correlation between the distribution of the streptomycin resistance gene (*strA*) and the taxonomic status of the streptomycin-producing type strains and natural isolates. Genotypic analysis gave the same results, whereby the natural isolates clustered with either the streptomycin-producing type strain *Streptomyces griseus* or with *Streptomyces griseoruber*. The streptomycin-resistance gene had not previously been detected in this group. This polyphasic approach demonstrated the usefulness of these methods for both taxonomic determination and the monitoring of gene flux in natural populations.

Ochi (1992) demonstrated that the mobility of ribosomal AT-L30 proteins on 2-D gels was genus-specific. This was further developed by sequencing and com-

relationships (b). The analysis was done using the following sequences from GenBank: D44283, *Streptomyces griseoviridis* DSM 40229^T; D44336, *Streptomyces fulvissimus* DSM 40593^T; D44025, *Streptomyces blastmyceticus* DSM 40029^T; D44289, *Streptomyces phaeochromogenes* ATCC 23945^T; D44282, *Streptomyces griseoruber* DSM 40281^T; D44234, *Streptomyces cyaneus* DSM 40108^T; D44174, *Streptomyces griseoflavus* DSM 40456^T; D44041, '*Streptomyces rochei*' JCM 4276; D44246, *Streptomyces fradiae* DSM 40063^T; D44218, *Streptomyces violaceus* DSM 40082^T; D44329, *Streptomyces diastaticus* subsp. *diastaticus* DSM 40496^T; D44143, *Streptomyces albidoflavus* DSM 40455^T; D44072, *Streptomyces chromofuscus* DSM 40273^T; D44004, *Streptomyces griseocarneus* DSM 40004^T; D44349, *Streptomyces hygroscopicus* subsp. *hygroscopicus* DSM 40578^T (cluster 32, *Streptomyces violaceusniger*); D44147, *Streptomyces albus* subsp. *albus* DSM 40313^T; D44184, *Streptomyces lydicus* DSM 40461^T; D44295, *Streptomyces rimosus* DSM 40260^T; D44191, *Streptomyces olivaceoviridis* DSM 40334^T; D44064, *Streptomyces atroolivaceus* DSM 40137^T; D44309, *Streptomyces anulatus* DSM 40361^T; D44083, *Streptomyces exfoliatus* 40060^T; D44293, '*Streptomyces lavendulae*' JCM 4664; D44247, *Streptomyces halstedii* ATCC 10897^T; D44364, *Amycolatopsis mediterranei* ATCC 13606^T. The phylogenetic analysis was performed using the DNADIST and NEIGHBOR programs of the PHYLIP (Felsenstein, 1993) software package. Bootstrap analysis were obtained from 1000 iterations, values over 600 are given. The bar represents a distance of 100.

Table 3. Taxonomic status of streptomycetes using genotypic and phenotypic characters

Streptomycete*	Cluster†
Group I	
S. anulatus ATCC 27416^T	A-1B
S. halstedii ATCC 19770^T	A-1C
S. aburaviensis (*Streptoverticillium olivoverticillatum*) ATCC 23869^T	A-2
S. californicus ATCC 19734^T	A-9
S. aureofaciens ATCC 23884^T	A-14
S. griseoruber ATCC 23919^T	A-21
S. microflavus ATCC 25474^T	A-23
S. graminofaciens ATCC 12705^T	A-26
S. violaceusniger ATCC 27477^T	A-32
S. noboritoensis (*S. chromogenes*) ATCC 25477^T	A-33
S. amakusaensis ATCC 23876^T	B
S. atroolivaceus ATCC 19725^T	A-3
S. badius ATCC 19888^T	C
S. lavendulae ATCC 19777^T	F-61
S. xanthochromogenes ATCC 19818^T	F-63
S. purpureus (*Kitasatoa purpurea*) ATCC 27787^T	F-65
S. psammoticus ATCC 25488^T	F-67
S. yerevanensis (*Microellobosporia violacea*) DSM 43167^T	D
S. fradiae ATCC 19760^T	G-68
S. finlayi ATCC 23906^T	I
Group II	
S. exfoliatus ATCC 19750^T	A-5
S. prunicolor ATCC 25487^T	A-11
S. phaeochromogenes ATCC 23945^T	A-40
S. pactum ATCC 27456^T	C-44
S. varsoviensis ATCC 25505^T	C-46
S. luridus ATCC 19782^T	F-62
S. bikiniensis ATCC 11062^T	F-64
S. lateritius ATCC 19913^T	H
Group III	
S. violaceus ATCC 25515^T	A-6
S. rochei ATCC 23956^T	A-12
S. chromofuscus ATCC 23896^T	A-15
S. griseoviridis ATCC 23920^T	A-17
S. olivaceoviridis ATCC 25478^T	A-20
S. poonensis (*Chainia poonensis*)	A-22
S. flaveolus ATCC 19754^T	A-24
S. viridochromogenes ATCC 14920^T	A-27
S. glaucescens ATCC 23622^T	A-28
S. filipinensis ATCC 23905^T	A-30
S. nogalater ATCC 27451^T	A-34
S. prasinosporus ATCC 17918^T	A-38
S. griseoluteus ATCC 12768^T	C-43
S. massasporeus ATCC 19785^T	D
S. fragilis ATCC 23908^T	G
Group IV	
S. alboflavus ATCC 12626^T	E-54
S. fulvissimus ATCC 27431^T	A-10
S. canus ATCC 19737^T	A-25
S. antibioticus ATCC 23879^T	A-31
S. aurantiogriseus ATCC 19887^T	A
S. novaecaesareae ATCC 27452^T	J
S. albidoflavus ATCC 25422^T	A-1A

Table 3 (*cont.*)

Streptomycete*	Cluster†
Non-grouped	
S. cellulosae ATCC 25439^T	A-13
S. albus ATCC 25426^T	A-16
S. diastaticus ATCC 3315^T	A-19
S. lydicus ATCC 25470^T	A-29
S. chattanoogensis ATCC 19739^T	A-35
S. thermovulgaris ATCC 25501^T	A-36
S. griseoflavus ATCC 25456^T	A-37
S. longisporoflavus ATCC 23932^T	A-39
S. ochraceiscleroticus (*Chainia ochracea*) ATCC 25478^T	A-41
S. rimosus ATCC 23955^T	B-42
S. aurantiacus ATCC 19822^T	C-45
S. turbercidicus ATCC 25502^T	C-47
S. bambergiensis ATCC 13879^T	A
S. ramulosus ATCC 19802^T	C
S. sulphureus ATCC 27468^T	C
S. misakiensis ATCC 23938^T	F-66

* Groupings obtained from the sequence comparison of the N termini of AT-L30 proteins (Ochi, 1995).

† Cluster groups as defined by Williams *et al*. (1983a). The species designations are according to Williams *et al*. (1989), names in parentheses represent invalidated genus/species names that were changed after the Williams *et al*. (1983a) publication.

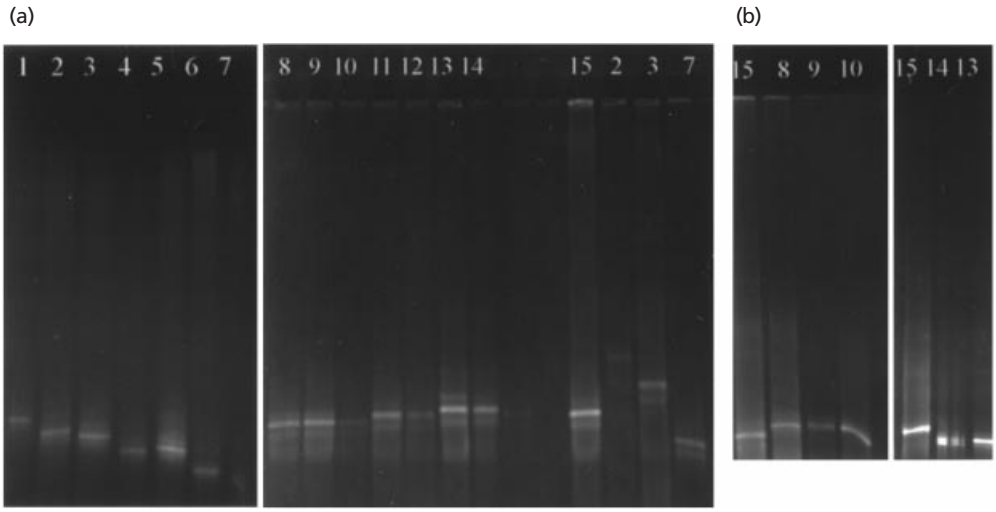

Fig. 4. DGGE separation of actinomycete DNA amplified using actinomycete-specific (a) and streptomycete-specific (b) PCR primers. Lanes: 1, '*Nocardiopsis albus*' DSM 43377; 2, *Micrococcus luteus* DSM 20030^T; 3, '*Arthrobacter crystallopoietes*' ATCC 15481; 4, *Streptosporangium roseum* ATCC 12428^T; 5, '*Streptomyces lividans*' TK21 (*Streptomyces griseoruber*, cluster 21); 6, *Saccharothrix* spp.; 7, '*Micromonospora citrea*' ATCC 3571; 8, *Streptomyces* sp. ASSF22 (cluster 1B); 9, *Streptomyces* sp. ASB33 (cluster 1B); 10, *Streptomyces griseus* DSM 40236 (cluster 1B); 11, *Streptomyces fluorescens* DSM 40203 (cluster 1B); 12, *Streptomyces coelicolor* Müller DSM 40233^T(cluster 1A); 13, *Streptomyces* sp. ASB37 (cluster 21); 14, '*Streptomyces lividans*' TK21 (*Streptomyces griseoruber*, cluster 21); 15, '*Streptomyces avermitilis*' ATCC 31267 (*Streptomyces diastaticus*, cluster 19).

paring the N termini of the protein from 81 streptomycetes (Ochi, 1995). Comparison of these sequences demonstrated that this method was phylogenetically coherent and was supported by 16S rRNA sequence analysis. There was some incongruency with the cluster groups as defined by phenetic groupings (Table 3), which may indicate some overspeciation within the streptomycetes as defined by phenotypic characters.

Further work is required to develop matrices that are coherent between phenetic tests and AT-L30 protein phylogeny, as this method clearly has the potential to be a useful taxonomic tool. Such studies must be done with identical strains because the use of strains bearing the same epithets but with different designated numbers has caused confusion in the past.

Rapid methods for gene analysis in streptomycete taxonomy

Alternative methods that do not involve sequencing are available for the taxonomic grouping of streptomycetes. Specific genes can be investigated for differences at the nucleotide level using either restriction analysis (Clarke *et al*., 1993; Fulton *et al*., 1995) or by monitoring the mobility of the product using specialized gel electrophoresis techniques (Heuer *et al*., 1997; Hain *et al*., 1997). RFLPs of rRNA can taxonomically differentiate species groups in yeasts (Laaser *et al*., 1989) and *Campylobacter* (Owen *et al*., 1989). Clarke *et al*. (1993) investigated their potential and definition for the *Streptomyces* genus using strains from the *Streptomyces albidoflavus* cluster (subgroups 1A and 1B). Purified rRNA was restricted using a combination of enzymes (*Bgl*I, *Eco*RI, *Pst*I and *Pvu*II). RFLP profiles were obtained after agarose gel electrophoresis. The patterns obtained varied between species groups and were sufficient for the differentiation of species, thus enabling phenotypically similar strains to be distinguished. Ribosomal restriction analysis was further developed by Fulton *et al*. (1995), *Mse*I fingerprints of rRNA operons (RiDiTS) were used to group 98 named streptomycete strains that included members of the phenotypic cluster groups A (subclusters 1–41) and F (subclusters 55–67) according to Williams *et al*. (1983a) and additional actinomycetes. RiDiTS revealed 11 pattern types, which had varying degrees of similarity to those defined by Williams *et al*. (1983a). Correlation was visible at low resolution (70 % similarity) but could not differentiate between the more defined phenotypic clusters. The method also correlated with 16S rRNA sequencing data, DNA–DNA hybridizations and whole-cell fatty acid analysis results. *Mse*I RiDiTS was successful at grouping streptomycetes taxonomically, but not at differentiating highly related strains.

Genotypic variation can also be monitored using either denaturing gradient gel electrophoresis (DGGE) (Muyzer *et al*., 1993) or DNA-binding agents (Hain *et al*., 1997) which alter the mobility of genes during electrophoresis according to nucleotide composition. DGGE can be used for the delimitation of genus-groups and species-groups when using the variable 16S rRNA regions (Fig. 4). Soil isolates ASB33, ASB37 and ASSF22 identified most closely to *Streptomyces albidoflavus*, *Streptomyces griseoruber* and *Streptomyces albidoflavus*, respectively, using a combination of techniques including numerical taxonomy (Huddleston, 1995), PFGE (Huddleston *et al*., 1995) and sequence comparisons (Huddleston *et al*., 1997). DGGE analysis using the variable γ region confirmed the taxonomic identification of these isolates (Fig. 4).

Identification of species – future directions

The purpose of this review has been to overview the current status of streptomycete taxonomy by tracing the different analytical methods that have been employed and the results that have been obtained. It is clear that the advent of molecular and genome analysis has had, and will continue to have, an important impact on the taxonomy of streptomycetes. New technologies such as automatic sequencers, pyrolysis mass spectroscopy and DGGE have been successfully applied, resulting in an increase in taxonomic data and improvement of discriminative analysis. As technology and automation improve it may soon be possible to combine data from the *Streptomyces coelicolor* A3(2) genome sequencing project generated at the Sanger Centre (UK) with DNA chip technology to create an automated method to further understand and categorize species groups within the *Streptomyces* genus. Thus, correlations between genotypic and phenotypic traits can be determined, resulting in a greater understanding of streptomycete taxonomy. This technology may have widespread availability, and information from the genome and proteome can be applied to the phenome, which has been so useful for taxonomy in the past. It is also worth considering the value of new technologies, while still maintaining traditional methodologies that still have value for specific applications, such as phenotypic identification of species using the probabilistic identification matrix. For a new taxonomic method to be validated, a universal standard selection of strains should be used by all groups doing these types of investigations. There should also be an increased emphasis on the methods used to analyse the data. Nucleic acid and protein sequences are being submitted to databases at an ever-increasing rate, and reliable numerical methods are required that can reproducibly analyse the enormous data sets that are being generated to allow critical discriminant analysis for identification procedures.

It is encouraging that despite the increasing applications of molecular techniques to streptomycete taxonomy, many of the clusters defined using numerical taxonomy are retained despite being positioned differently; this is illustrated by the *Streptomyces albidoflavus* subclusters (Figs 1 and 3a, b). A greater emphasis needs to be placed on which phenotypic traits can be used. Certain phenotypes, such as antibiotic resistance, antibiotic production and pathogenicity, cannot be used because they might be the result of a horizontal gene-transfer event (Anderson *et al*., 2000; Bukhalid *et al*., 1998). Identification systems should also be inexpensive, user friendly and reproducible. The method of choice is also influenced by the application for which the identification is required. Some applications, for example patenting and strain

deposition, only require identification to the genus level, whereas others, such as epidemiological and evolutionary studies, require identification to the subspecies level. Ecological investigations and isolation for screening purposes need to monitor diversity, so as to remove duplicates. For this, species evaluation may not be enough, because many phenotypes such as secondary-metabolite production are not species-dependent, and discarding isolates at the species level may be detrimental. For example, '*Streptomyces avermitilus*' produces the antiparasitic compound avermectin and was identified to the *Streptomyces diastaticus* cluster (cluster 19) (Williams, 1995). Production of this compound has never been detected in any other organism, underlying the importance of devising methods to distinguish between species and strains. This emphasizes the importance of a polyphasic taxonomic approach, which can only be maintained by standardization and collaboration so that molecular, biochemical and phenotypic traits can be weighted and examined in an integrative manner. Standardization is also required for the phenotypic and biochemical techniques to provide reliable identification procedures that can be updated to keep pace with new sequence data and other molecular and biochemical analyses.

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