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Biosystematics of alkaliphilic streptomycetes isolated from seven locations across a beach and dune sand system

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Abstract Alkaliphilic streptomycetes were isolated from composite sand samples collected from six out of seven locations across a beach and dune sand system using starch-casein-nitrate agar supplemented with cycloheximide and buffered to pH 10.5. The isolates had colonial and chemotaxonomic properties consistent with their classification in the genus *Streptomyces*. They were assigned to 49 multimembered and 114 single-membered colour-groups given their ability to produce pigments on oatmeal and peptone-yeast-extract-iron agars and to corresponding taxa based on whole-genome *rep*-PCR banding patterns. Twenty-four isolates representing the colour and *rep*-PCR groups grew well from pH 5 to 11, and optimally at pH 9, as did phylogenetically close members of the *Streptomyces griseus* 16S rRNA gene clade. One hundred and twelve representative alkaliphilic streptomycetes formed a heterogeneous but distinct clade in the *Streptomyces* 16S rRNA gene tree. A 3-dimensional representation of 16S rRNA sequence data showed that the alkaliphilic streptomycetes formed a distinct group in multidimensional taxospace. It is evident that alkaliphilic streptomycetes are common in the beach and dune sand system and that representatives of this community form new centers of taxonomic variation within the genus *Streptomyces* that can be equated with species.

Keywords Alkaliphilic streptomycetes · pH profiles · Polyphasic taxonomy · Selective isolation

Introduction

Genotypic and phenotypic methods are being used to clarify relationships within the genus *Streptomyces* (Goodfellow et al. 1992; Manfio et al. 1995; Anderson and Wellington 2001; Goodfellow et al. 2007). 16S rRNA gene sequence data show that type strains of many *Streptomyces* species can be assigned to multimembered species-groups, as exemplified by the *S. albidoflavus* (Manfio et al. 1995; Lanoot et al. 2005), *S. griseus* (Liu et al. 2005), *S. violaceoruber* (Duangmal et al. 2005) and *S. violaceusniger* clades (Kumar and Goodfellow 2008). Nevertheless, the genus *Streptomyces* remains underspeciated (Sembiring et al. 2000; Manfio et al. 2003; Kumar and

GenBank accession numbers for the 16S rRNA gene sequences for the strains of the alkaliphilic streptomycetes Bd 095, Bd 064, Bd 077, Bd 013, Bd 108, Bd 088, Bd 012, Bd 187, Bd 128, Bd 174, Bd 167, Lt 005, Lt 006, Fd 015, Bd 099, Bd 059, Bd 159, Ht 015, Md 005, Ht 020, Bd 205, Md 063, Fd 004, Md 039 and Bd 092 are EU477215, EU477216, EU477217, EU477218, EU477219, EU477220, EU477221, EU477222, EU477223, EU477224, EU477225, EU477226, EU477227, EU477228, EU477229, EU477230, EU477231, EU477232, EU477233, EU477234, EU477235, EU477236, EU477237, EU477238 and EU477257, respectively.

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Goodfellow 2008), partly because biosystematic studies have been directed towards unravelling relationships between mesophilic, neutrophilic streptomycetes which, in culture, grow between pH 6.5 and 8.0 (Pridham and Tresner 1974), with an optimum around 7.0 (Mikami et al. 1982; Williams et al. 1984; Manfio et al. 1995). In contrast, relatively little attention has been given to determining the taxonomic diversity of acidophilic, alkaliphilic or neutrotolerant streptomycetes.

Neutrotolerant streptomycetes, which grow from pH 3.5 to 7.5, form a taxonomically diverse group, the members of which are common in acidic habitats (Williams et al. 1971; Kim et al. 2004; Xu et al. 2006), as are their strictly acidophilic counterparts which have a more restricted pH range (Williams et al. 1971; Goodfellow and Dawson 1978; Seong et al. 1993). Mikami et al. (1982) distinguished between alkaliphilic actinomycetes, which grew between pH 8.0 and 11.5, with optimal growth around pH 9.0–9.5, from alkaline-resistant actinomycetes which grew at high pH values but optimally at pH 7.0. Alkaliphilic and alkalitolerant streptomycetes are common in alkaline soils (Taber 1960; Basilio et al. 2003; Antony-Babu et al. 2007), but few such organisms have been formally described as novel *Streptomyces* species (Kim et al. 1999; Huang et al. 2004; He et al. 2005; Hozzein et al. 2008; Mao et al. 2007).

Streptomycetes remain a rich source of new secondary metabolites (Ōmura et al. 2001; Watve et al. 2001; Bentley et al. 2002; Ikeda et al. 2003; Strohl 2004; Bérdy 2005; Fiedler et al. 2005) hence the continued interest in screening them for novel bioactive compounds (Bérdy 2005; Fiedler et al. 2005). However, it is becoming increasingly difficult to discover commercially useful secondary metabolites from common streptomycetes as this practice leads to the rediscovery of known bioactive compounds thereby emphasising the need to isolate, characterise and screen novel members of the genus. Streptomycetes from un- and under-explored habitats are proving to be a rich source of new bioactive compounds, including antibiotics (Bull et al. 2005; Fiedler et al. 2005).

It is timely to selectively isolate and determine the taxonomic diversity of alkaliphilic streptomycetes as these organisms are a useful source of new bioactive compounds (Solingen et al. 2001; Dieter et al. 2003; Höltzel et al. 2003; Bruntner et al. 2005; Mehta et al. 2006; Vasavada et al. 2006; Graf et al. 2007). The primary aim of the present study was to determine the numbers and taxonomic diversity of putative alkaliphilic streptomycetes isolated from composite sand samples collected across a beach and dune sand system and to establish the pH profiles of representative isolates and phylogenetically related streptomycetes.

Materials and methods

Site and sampling

Dune and beach sand samples were collected from seven locations across a ten foot transect along the beach and dune sand system at Ross Links, County Northumberland, United Kingdom (National Grid Reference NU 1452038351). The fore-dunes were colonized by sea wheat grass (*Thinopyrum junceiforme* [Á. Löve & D. Löve] Á. Löve & D. Löve) and the mid- and back-dunes by European beach grass (*Ammophila arenaria* [L.] Link). Samples were collected in May 2003 from the low water mark to the fixed *Ammophila* dunes from a depth of about 10 cm below the surface to minimize the influence of mobile surface sand. The samples were transported to the laboratory in an insulated container at 4°C and stored at −20°C. Four samples from each location were thoroughly mixed to make composite samples.

Physical and chemical properties of the samples

The bulk pH of samples collected at each sampling site were determined following the procedure described by Reed and Cummings (1945) using a glass electrode pH meter (Model 320, Mettler-Teledo, Schwerzenbach, Switzerland). Each sample was examined in triplicate and the final pH values recorded as an average of the three readings. Similarly, percentage moisture contents of triplicate samples were established by drying known weights of sand at 105°C to constant weight and calculating the average values. The dried samples were placed in a muffle furnace (Carbolite, Sheffield, England, UK) and the temperature raised slowly to 700°C and kept constant for 30 min to burn off organic matter. After cooling overnight in a desiccator, the average loss in weight for three readings was recorded

as the organic matter content. Total carbon contents in the samples were estimated using the procedure recommended by the British Standards Institution (1995).

Selective isolation and enumeration of presumptive streptomycetes

One gram wet weight of each composite sample was suspended in 9 ml of ¼ Ringer's solution (Oxoid) in a universal bottle. The resultant 10^{-1} preparations were agitated on a shaker (Gallenkamp Orbital Incubator, Loughborough, United Kingdom) at 150 revolutions per minute (rpm) for 10 min at room temperature, heated at 55°C for 6 min in a water bath, and cooled at room temperature. The resultant preparations were serially diluted down to 10^{-6}, using ¼ strength Ringer's solution, and aliquots of each dilution (100 µl) spread over the surfaces of dried starch-casein agar plates (Kűster and Williams 1964) supplemented with cycloheximide (50 µg/ml) and adjusted to pH 10.5 with NaOH; the plates had been dried for 15 min prior to inoculation, as recommended by Vickers et al. (1984). The inoculated plates, 5 per dilution per composite sample, were incubated at 28°C for 2 weeks. Colonies of presumptive alkaliphilic streptomycetes recognized by their ability to form leathery colonies and an aerial spore mass were counted and expressed as the number of colony forming units (cfu) per gram dry weight sand, as were presumptive non-streptomycete actinomycetes.

Selection, maintenance and initial identification of isolates

Three hundred and twenty-one representative colonies putatively assigned to the genus *Streptomyces* were randomly selected from the starch-casein isolation plates and subcultured onto oatmeal agar plates (ISP 3; Shirling and Gottlieb 1966) which were incubated at 28°C for 14 days. Purified isolates were maintained on oatmeal agar slopes (Shirling and Gottlieb 1966) and as suspensions of spores and hyphal fragments in glycerol (20%, v/v) at −20°C. The isolates were examined for the presence of isomers of diaminopimelic acid (A_2pm) in whole-organism hydrolysates using the procedure described by Staneck and Roberts (1974). A standard solution (10 µM) of A_2pm (Sigma) containing a mixture of *DL*-, *LL*- and *meso*-A_2pm isomers was used as a reference. One hundred and six representatives of other actinomycete colonies were selected from the isolation plates, grown on modified Bennett's agar (Jones 1949), and presumptively identified to the genus level using morphological, chemotaxonomic and 16S rRNA gene sequence data.

Dereplication of isolates

The 321 representative isolates were assigned to the genus *Streptomyces* as they gave whole-organism hydrolysates rich in the *LL*-isomer of diaminopimelic acid and formed leathery colonies covered by an abundant aerial spore mass. They were inoculated onto oatmeal (ISP 3; Shirling and Gottlieb 1966) and peptone-yeast extract-iron agar (ISP 6; Shirling and Gottlieb 1966) plates which were incubated at 28°C for 14 and 4 days, respectively. The oatmeal agar plates were examined by eye to determine aerial spore mass colour, substrate mycelial pigmentation and the colours of any diffusible pigments, using National Bureau of Standards (NBS) Color Name Charts (Kelly 1964), and the peptone-yeast extract-iron agar plates examined for the production of melanin pigments. The isolates were assigned to colour-groups based on pigments produced on these media.

The isolates were also assigned to *rep*-PCR molecular fingerprint groups. To this end, DNA isolation and electrophoresis were carried out on biomass harvested at 30°C for 4 days on a medium consisting of 1%, w/v glucose, 1%, w/v yeast extract, 0.5%, w/v beef extract and 1.5%, w/v agar. DNA extraction was carried out following an established procedure (Kieser et al. 2000) and the *rep*-PCR with the BOX A1R primer, as described by Versalovic et al. (1991). Band patterns were analysed with the Pearson's product moment correlation coefficient (*r*-value) (Häne et al. 1993) and the unweighted pair group method with the arithmetic averages algorithm (UPGMA), using BIONUMERICS software (Applied Maths, Belgium).

Sequencing of 16S rRNA genes

Isolation of chromosomal DNA, PCR and direct sequencing of 16S rRNA genes of 128 isolates taken to represent the multimembered colour and molecular fingerprint groups was carried out using a standard procedure (Duangmal et al. 2005). The resultant 16S

rRNA gene sequences were aligned manually against corresponding sequences of available type strains of *Streptomyces* species, retrieved from GenBank, using PHYDIT software (Chun 1995). An evolutionary distance matrix of the 16S rRNA gene sequences was generated as described by Jukes and Cantor (1969) and the topology of the resultant tree evaluated in a bootstrap analysis (Felsenstein 1985) based on 1,000 resamplings, using the TREECON W program (Van de Peer and De Wachter 1994). A principal coordinate analysis based on the distances between the 16S rRNA gene sequences of 35 representative isolates and 55 members of previously established *Streptomyces* clades was carried out using NTSYS software (Rohlf 1988). This analysis was performed by generating eigenvectors of double center calculated for the distances; the resultant coordinates were visualized using the XLSTAT program (Fahmy and Aubry 2003).

Determination of pH profiles

The pH profiles of 24 isolates chosen to represent subclades in the *Streptomyces* 16S rRNA gene tree were determined together with those of *Streptomyces griseus* strains DSM 40226, DSM 40307, DSM 40395, DSM 40236^T and DSM 41811, and *S. sanglieri* DSM 41791^T (Liu et al. 2005). The organisms were grown on oatmeal agar (Kűster and Williams 1964) plates for 14 days at 28°C when spores were harvested by scrapping them from agar surfaces. The resultant spore preparations were each suspended in sterile distilled water and washed by centrifugation at 14,000 rpm for 10 min and the supernatants discarded (Kieser et al. 2000). This procedure was repeated five times and the resultant washed spore suspensions (20 µl) pipetted into two 1.5 ml microfuge tubes each of which contained 500 µl of peptone-yeast extract broth (Kieser et al. 2000) adjusted at unit intervals from pH 4 to 12, using either NaOH or HCl. These preparations were made in duplicate, and the tubes sealed with parafilm and incubated at 28°C in an horizontal shaker (Gallenkamp Orbital Incubator) at 220 rpm.

Individual tubes removed from the shaker at two hourly intervals following incubation of each strain for 10–46 h were instantly frozen to −80°C to prevent further growth. The resultant preparations were centrifuged, and the pelleted cells suspended in 500 µl of sterile distilled water and recentrifuged; this process was repeated five times when the washed pellets were resuspended in 500 µl of ¼ strength Ringer's solution. The OD_{600} of the cell suspensions of the individual preparations were taken to represent the growth density of each of the strains at that specific time. The means of the duplicated preparations were plotted as growth profile graphs using Microsoft EXCEL software. The pH values which showed the maximum growth of each strain in minimum time were recorded as the optimal growth pH values.

Results

Growth profiles

The 24 isolates taken to represent the colour and *rep*-PCR groups and the six *S. griseus* strains grew well from pH 6 to 10, with moderate growth at pH 5, as exemplified in Fig. 1. All of these strains showed optimal growth at pH 9 within 40 h. These data are consistent with the classification of the isolates and the representatives of the *S. griseus* clade as alkaliphilic streptomycetes. In contrast, the "*S. coelicolor*" A3(2) grew well from pH 6.0 to 9.0, and optimally at pH 7.0 (Fig. 1).

Selective isolation and enumeration

The 321 isolates gave whole-organism hydrolysates rich in *LL*-A_2pm thereby confirming that they were members of the genus *Streptomyces*. The physicochemical properties of the samples and the numbers of alkaliphilic streptomycetes and actinomycete-like isolates are shown in Table 1. It is evident that the highest number of alkaliphilic streptomycetes, namely 32×10^6 cfu's per gram dry weight sand, was found in the back-dune sample; these organisms accounted for 44% of the total number of alkaliphilic streptomycetes. In contrast, the lowest number of alkaliphilic streptomycetes, 1.5×10^5 cfu's per gram dry weight sand, was found in the low beach sample. Alkaliphilic streptomycetes were not isolated from the middle beach sample. Preliminary studies based on morphology, presence of the *meso*-A_2pm and 16S rRNA phylogeny indicated that most of the non-streptomycete actinomycetes growing on isolation

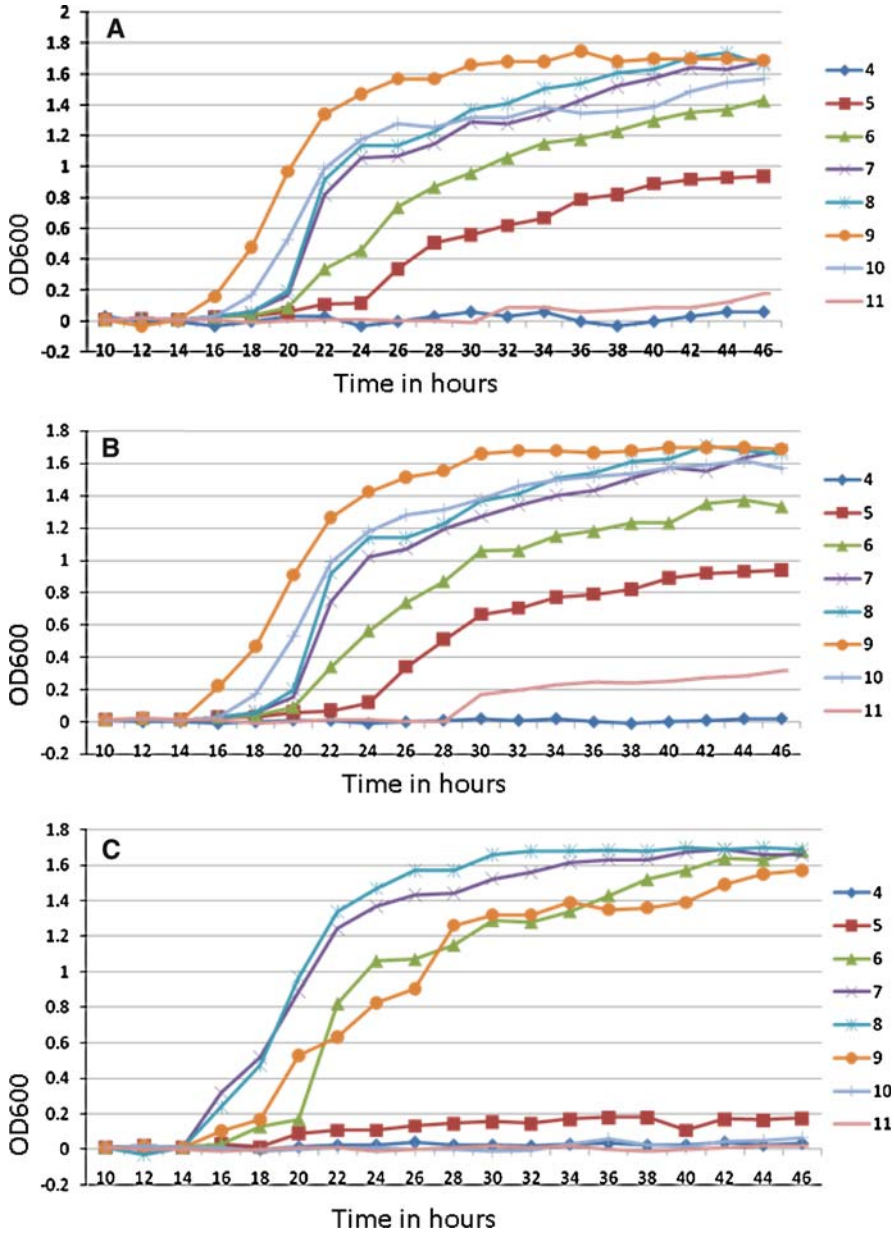

Fig. 1 Growth profiles of (**a**) alkaliphilic streptomycete isolate Bd 205, (**b**) *Streptomyces griseus* DSM 40236^T and (**c**) "*Streptomyces coelicolor*" A3(2)

plates were members of the genera *Cellulosimicrobium*, *Rhodococcus* and *Tsukamurella*.

Dereplication

The 321 alkaliphilic streptomycetes were assigned to 151 colour-groups (Supplementary data, Table 1) composed of 18 major (5–11 isolates), 31 minor (2–4) and 114 single-membered taxa (Table 2). All of the major-colour groups and most of the minor and single-membered ones were composed of strains isolated from the mid- and back-dune composite sand samples. In contrast, relatively few isolates were from the beach and fore-dune composite sand samples. The genomic fingerprints derived from the repetitive sequence based PCR reactions using the BOX-PCR primer consisted of between 3 and 28 bands as shown in Fig. 2. Fragment sizes ranged from about 100 to 2500 base pairs though resolution of sizes below 100 bp was limited. Bands within individual fingerprints showed varying degrees of intensity (Fig. 2). The isolates were assigned to 166 *rep*-PCR groups. Excellent congruence was found

Table 1 Physicochemical properties and numbers of alkaliphilic streptomycetes and actinomycete-like strains isolated from seven locations across the beach and dune sand system at Ross Links, County Northumberland

Sampling site	pH	Total carbon (%)	Organic carbon (%)	Numbers of alkaliphilic streptomycetes per gram dry weight sand ($\times 10^5$)	Numbers of actinomycete-like isolates per gram dry weight sand ($\times 10^5$)
Beach					
Low	7.4	1.4	0.8	1.5	12
Middle	7.6	2.2	0.5	0	0.00004
Upper	7.6	3.2	0.5	2.6	2.6
Seasonal upper	8.0	3.1	0.7	2.9	2.9
Dunes					
Fore-dune	8.0	2.9	1.1	5.2	5.2
Mid-dune	6.8	1.5	1.3	18	21.8
Back-dune	7.2	1.5	1.3	32	42.1

Table 2 Numbers of major, minor and single-membered colour-groups recorded from each of the sampling sites

Sites	Colour groups			Total number of colour groups per site
	Major	Minor	Single-membered	
Beach				
Low	0	2	4	6
Middle	0	0	0	0
Upper	0	4	12	16
Seasonal upper	0	0	28	28
Dunes				
Fore-dune	0	3	19	22
Mid-dune	10	4	6	20
Back-dune	8	18	45	71
Total number of colour groups	18	31	114	163

between the composition of the colour and BOX-PCR groups as 301 out of the 321 isolates (94%) were assigned to matching groups. The exceptions are accounted for by three colour groups in the BOX-PCR fingerprint analysis split into seven smaller groups.

16S rRNA gene sequences

One hundred and twelve out of the 128 representative alkaliphilic streptomycetes (88%) formed a distinct clade in the 16S rRNA gene tree together with the representatives of the *S. griseus* clade, the remaining alkaliphilic streptomycetes were assigned to a second clade. Representatives of each of these clades populated previously unoccupied taxospace within the evolutionary radiation of members of the genus *Streptomyces*, as illustrated in Fig. 3. Representatives of the main group of alkaliphilic streptomycetes formed an heterogeneous group that was most closely related to members of the *S. griseus* 16S rRNA gene clade (Fig. 4).

Discussion

The results of the present study confirm and extend those from previous investigations (Mikami et al. 1982; Basilio et al. 2003) by showing that some soil streptomycetes grow well over a wide pH range, and optimally at pH 9.0. These are interesting results as nearly all *Streptomyces* type strains grow over a restricted pH range and optimally at pH 7.0 (Mikami et al. 1982), exceptions include the type strains of *S. glauciniger* (Huang et al. 2004), *S. jietaisiensis* (He et al. 2005), *S. radiopugnans* (Mao et al. 2007), "*S. sannurensis*" (Hozzein et al. 2008) and those of the thermophiles *S. thermoalcalitolerans*, *S. thermocarboxydovorons*, *S. thermodenitrificans* and *S. thermovulgaris* all of which grow over an extensive pH range (Kim et al. 1999). This list can be extended to include authenticated *S. griseus* strains (Liu et al. 2005) as representatives of this taxon were shown to grow optimally at pH 9.0.

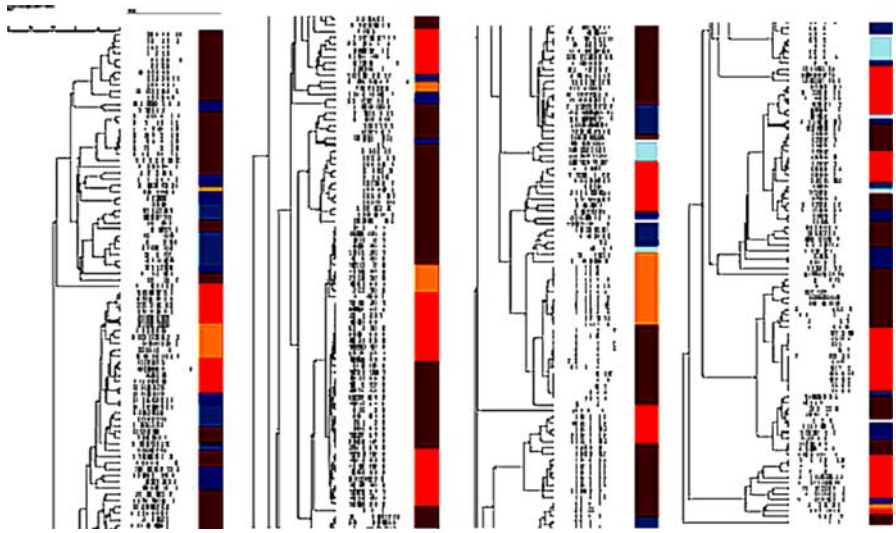

Fig. 2 Pearson-UPGMA cluster analysis of BOX-PCR fingerprints of alkaliphilic streptomycetes. The colour bars denote the composite sand sample from which the strains were isolated: Back-dune ■, Mid-dune ■, Fore-dune ■, seasonal upper beach ■, upper beach ■ and low beach ■

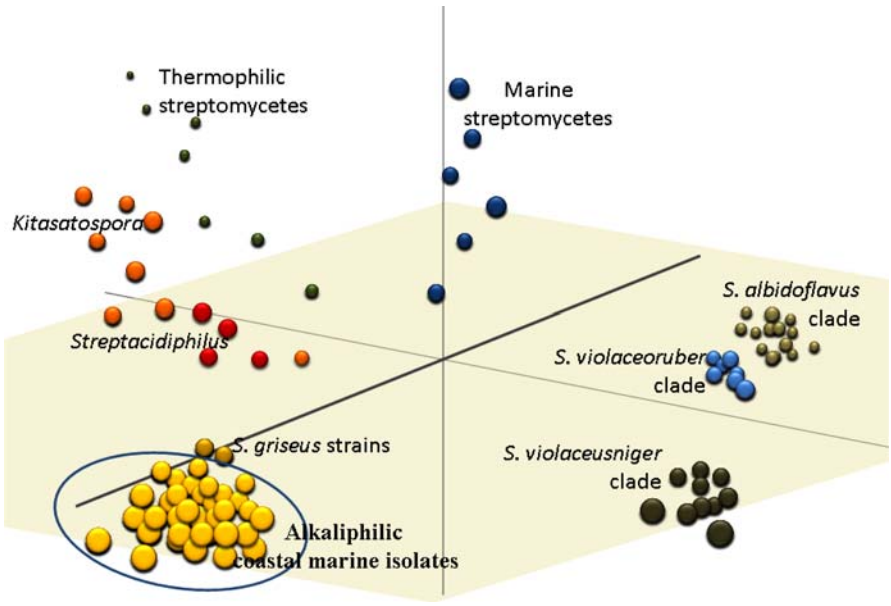

Fig. 3 Taxonomic space occupied by representative alkaliphilic streptomycetes, 43 representatives of the *S. albidoflavus*, *S. griseus*, *S. violaceoruber* and *S. violaceusniger* clades and members of the genera *Kitasatospora* and *Streptacidiphilus* based on 16S rRNA gene sequence data. Members of the *Streptomyces* clades: composition of *Streptomyces* clades (a) *S. albidoflavus*: *S. albidoflavus* species strains DSM 40455^T, DSM 40867, DSM 40880, DSM 41186, DSM 41812, DSM 41816 and DSM 46452, *S. anandii* DSM 40535^T, *S. canescens* DSM 40001^T, *S. eurythermus* DSM 40014^T, *S. felleus* DSM 40130^T, *S. intermedius* DSM 40372^T, *S. odorifer* DSM 40347^T and *S. sampsonii* DSM 40394 ^{T}, (b) *S. griseus*: *S grisues* DSM 40236^T and *S. yanii* DSM 43887^T, (c) *S. violaceoruber*: "*S. caesius*" NRRL B-12000, *S. coelescens* NRRL B-12348^T, "*S. coelicolor*" A3(2), *S. humiferus* NRRL B-3088^T, "*S. lividans*" NRRL B-16637, *S. tendae* ATCC 19812^T and *S. violaceoruber* NRRL B-3319^T and (d) *S. violaceusniger*: *S. asiaticus* DSM 41761^T, *S. cangkringensis* DSM 41769^T, *S. hygroscopicus* NRRL 1477, *S. indonesiensis* DSM 41759^T, *S. javensis* DSM 41764^T, *S. malaysiensis* DSM 41697^T, *S. melanosporofaciens* NRRL B-12234^T, *S. rhizosphaerius* DSM 41760^T, *S. violaceusniger* NRRL B-1865 and *S. yogyakartensis* DSM 41766^T

Presumptive alkaliphilic streptomycetes were isolated from six out of the seven composite sand samples taken along the transect at Ross Links beach and dune sand system using heat preheated suspensions of sand and starch-casein agar supplemented with cycloheximide and adjusted to pH 10.5. Initial

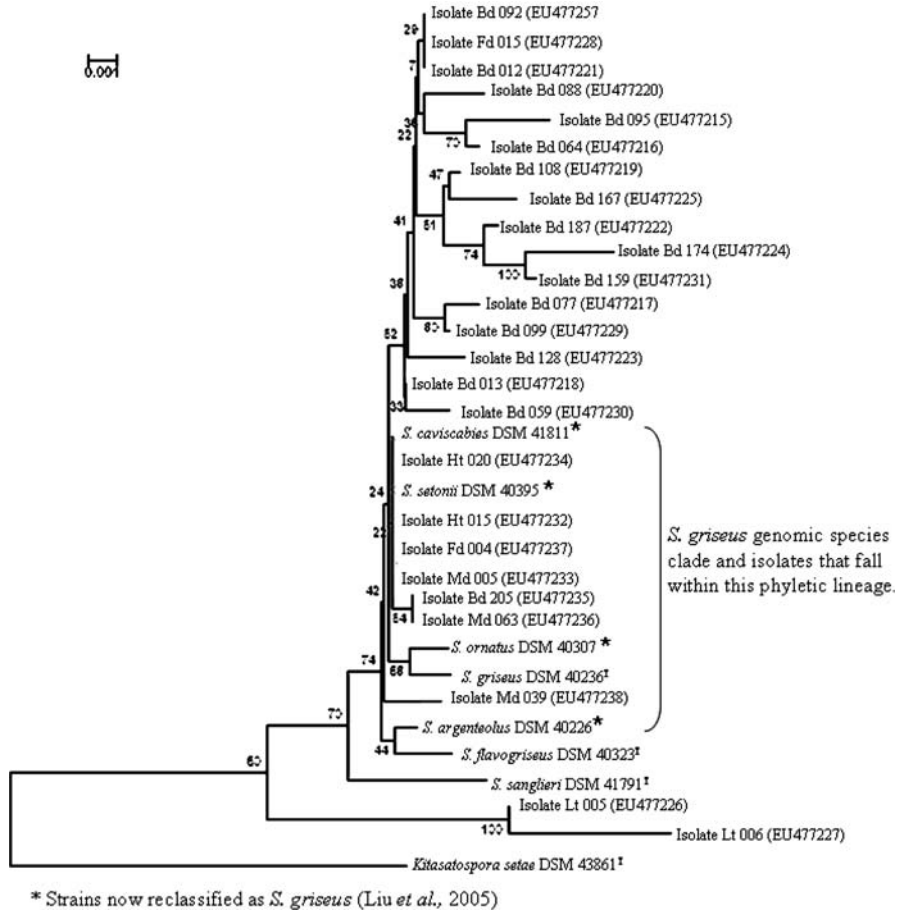

Fig. 4 Neighbor-joining tree (Saitou and Nei 1987) based on nearly complete 16S rRNA gene sequences showing relationships between representative alkaliphilic streptomycetes and phylogenetically close relatives belonging to the *S. griseus* clade. Numbers at the nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1,000 resampled datasets. Bar, 0.001 nucleotide substitutions per nucleotide position

studies showed that all of the presumptive streptomycetes taken from the isolation plates belonged to the genus *Streptomyces* as they formed extensively branched substrate mycelia, an aerial spore mass, and gave whole-organism hydrolysates rich in *LL*-A_2pm (Manfio et al. 1995). In addition, the selected isolates grew well from pH 6.0 to 10, and optimally at pH 9.0; all of these organisms were assigned to the *Streptomyces* 16S rRNA gene clade. These results confirm and extend those from previous studies in showing that alkaliphilic streptomycetes are common in soil (Mikami et al. 1982; Basilio et al. 2003).

Distinct quantitative and qualitative differences were observed between the alkaliphilic streptomycetes recovered from the various composite sand samples. In general, the numbers and types of these organisms were low in the beach sand and fore-dune samples rising markedly in the mid- and back-dune sand samples covered by higher plants, notably by *A. arenaria*. These findings are in good agreement with those reported for the two beach and dune sand systems studied by Watson and Williams (1974).

It was particularly interesting that six alkaliphilic streptomycetes showed >99% 16S rRNA gene similarities to *S. griseus* DSM 41811 and hence are *bona fide* members of *S. griseus* (Liu et al. 2005). These strains were isolated from different sand samples. Isolates Bd 205, Fd 004 and Ht 020 were derived from back-dune, fore-dune and upper-beach composite sand samples, respectively and isolates Md 005, Md 039 and Md 063 from the mid-dune sample. These observations suggest that *S. griseus* strains have a cosmopolitan distribution in line with the Bass Becking hypothesis that "everything is everywhere" (Hedlund and Staley 2004).

The taxonomic diversity encompassed by streptomycetes is extraordinary as new and putatively novel *Streptomyces* species are being isolated from neglected habitats, including rhizosphere soil (Goodfellow et al. 2007; Kumar and Goodfellow 2008) and marine

sediments (Goodfellow and Haynes 1984). It is evident from the 16S rRNA gene sequence data that many of the alkaliphilic streptomycetes isolated from the beach and dune sand system at Ross Links form a novel heterogeneous group. This point is underlined by the assignment of the isolates to 49 multimembered and 114 single-membered colour-groups as it has been shown repeatedly that streptomycete groups based on aerial spore mass, colony reverse and diffusible pigment colours on oatmeal agar, and on the formation of melanin pigments on peptone-yeast extract-iron agar are predictive; strains taken to represent such taxa key out to either previously described or novel *Streptomyces* species based on computer-assisted identification (Goodfellow and Haynes 1984; Williams and Vickers 1988; Atalan et al. 2000), Curie-point pyrolysis mass spectrometric (Atalan et al. 2000) and polyphasic taxonomic procedures (Manfio et al. 2003). In general, members of the multimembered colour groups were isolated from single locations suggesting that they may be spatially distributed across the beach and dune sand system.

The present findings not only show that alkaliphilic streptomycetes are common in moderately alkaline soils but provide an invaluable basis for determining the taxonomic variation, distribution and roles of these organisms in such habitats. They also have clear implications for bioprospecting, not least because alkaliphilic streptomycetes are proving to be a rich source of bioactive compounds, as exemplified by the production of frigocyclinone, a novel angucyclinone antibiotic from *S. griseus* NTK 97 (Bruntner et al. 2005), lactonamycin Z, a new antibiotic and antitumor compound from *S. sanglieri* AK 623 (Höltzel et al. 2003), and elloxazinones A and B, two new aminophenoxazinone compounds from *S. griseus* Acta 2871 (Graf et al. 2007).

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