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Streptomyces alkaliterrae* sp. nov., isolated from an alkaline soil, and emended descriptions of *Streptomyces alkaliphilus*, *Streptomyces calidiresistens* and *Streptomyces durbertensis

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Abbreviations: A₂pm, diaminopimelic acid; ANI, average nucleotide identity; BGCs, biosynthetic gene clusters; BLAST, Basic Local Alignment Search Tool; dDDH, digital DNA–DNA hybridization; DDH, DNA–DNA hybridization; DNA, deoxyribonucleic acid; GGDC, genome to genome distance calculator; ISP, International *Streptomyces* Project; MIDI, Microbial Identification System; MK, menaquinones; NCIMB, National Collection of Industrial, Food and Marine Bacteria; PCM, Polish Collection of Microorganisms; RAST, Rapid Annotation using Subsystem Technology; rRNA, ribosomal RNA; S., *Streptomyces*; TLC, thin-layer chromatography.

Abstract

A polyphasic study was undertaken to establish the taxonomic position of six representative streptomycetes isolated from an alkaline soil adjacent to a meteoric alkaline soda lake in India. Chemotaxonomic, cultural and morphological properties of the isolates were consistent with their classification in the genus *Streptomyces*. The isolates formed extensively branched substrate mycelia and aerial hyphae that differentiated in straight chains of spores with smooth surfaces. They contained *LL*-diaminopimelic acid in the wall peptidoglycan, produced either hexa- or octa-hydrogenated menaquinones with nine isoprene units, major amounts of saturated, *iso*- and *anteiso*- fatty acids and phosphatidylethanolamine as the characteristic polar lipid. The isolates grew well at 30 °C, pH 9 and in the presence of 3 to 5% (w/v) sodium chloride. Isolates OF1^T, OF3 and OF8 formed a distinct clade within the *Streptomyces* 16S rRNA gene tree sharing relatively high sequence similarities with the type strains of *Streptomyces durbertensis* (99.3%), *Streptomyces palmae* (98.1%) and *Streptomyces xinghaiensis* (98.3%), but can be distinguished from them using combinations of phenotypic properties. A phylogenomic tree based on draft genome sequences of the isolates and *S. durbertensis* DSM 104538^T confirmed the phylogenetic relationships. Average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values calculated from the whole genome sequences of isolate OF1^T and *S. durbertensis* DSM 104538^T were low at 92.0% and 45.2%, respectively, indicating that they belong to different genomic species. Consequently, on the basis of the genomic, phylogenetic and associated phenotypic data it is proposed that isolates OF1^T, OF3 and OF8 be assigned to the genus *Streptomyces* as *Streptomyces alkaliterrae* sp. nov. with strain OF1^T (NCIMB 15195^T =PCM 3001^T) as the type strain.

Isolates IF11, IF17 and IF19, and *S. alkaliphilus* DSM 42118^T were shown to belong to the same taxospecies and together with *S. calidiresistens* DSM 42108^T comprised a well supported clade in the *Streptomyces* 16S rRNA gene tree. Isolate IF17 and *S. alkaliphilus* DSM 42118^T formed a well-supported clade in the phylogenomic tree, had almost identical digital G+C similarity values, produced long straight chains of smooth-surfaced spores and shared ANI and dDDH values (98.0 and 79.6%, respectively) consistent with their assignment to the same genomic species. In light of all of the data isolates IF11, IF17 and IF19 should be seen as authentic strains of *S. alkaliphilus*. Data acquired in the present study have also been used to emend the descriptions of *S. alkaliphilus*, *S. calidiresistens* and *S. durbertensis*. The genomes of isolates IF17, and OF1^T, OF3 and OF8 contain relatively high numbers of biosynthetic gene clusters some of which were discontinuously distributed indicating ones predicted to express for novel specialised metabolites.

Keywords: Alkaliphilic streptomyces; Polyphasic taxonomy; Genomics; *Streptomyces alkaliterrae*; Phylogeny; Lonar Lake soil.

Introduction

Novel filamentous actinobacteria with genomes rich in biosynthetic gene clusters *sensu* Baltz [10] are a key element in the search for new drugs needed in the fight against multi-drug resistant microbial pathogens [31]. Novel neutrophilic streptomycetes (optimal growth around pH 7.0) from deep sea sediments and desert soils are a productive source of new bioactive compounds [15,37,60,65] but, in contrast to these microorganisms, alkaliphilic streptomycetes (optimal pH 9.0) [4] have rarely featured in bioprospecting campaigns despite their biotechnological potential [34]. The realisation that alkaliphilic streptomycetes have a pronounced capacity to synthesize new bioactive compounds [63,71] sparked renewed interest in them leading to the recognition of new species, such as *Streptomyces alkaliphilus* [1], *Streptomyces durbertensis* [86] and *Streptomyces lonarensis* [68] and to the discovery that an alkaliphilic streptomycete inhibits the growth of multi-drug resistant ESKAPE pathogens [78].

Alkaliphilic streptomycetes are common in alkaline soils and soda lakes [70,76], form a distinct taxon in multi-dimensional taxospace [3] and are markedly underspeciated [5]. The discovery that alkaliphilic streptomycetes isolated from sites across a beach and dune sand system could be assigned to *Streptomyces griseus* ecovars was seen to have implications for bioprospecting [4], as do extensive biogeographical studies on global communities of streptomycetes [2,66]. Improved matrices for the recognition of boundaries between prokaryotic species, subspecies and infrasubspecific taxa [9,20,48,55] are bringing greater precision to such studies.

The present investigation was designed to establish the taxonomic status of alkaliphilic streptomycetes isolated from an alkaline soil in India and to show whether representative isolates have the potential to synthesise novel antibiotics. The isolates and the type strains

of closely related *Streptomyces* species were the subject of a polyphasic study which drew upon data from draft whole genome sequences of representative strains. Some of the isolates were shown to be members of *S. alkaliphilus* [1] while others formed a new centre of taxonomic variation in the genus *Streptomyces*. The name proposed for this new taxon is *Streptomyces alkaliterrae* sp. nov., with isolate OF1^T as the type strain.

Materials and methods

Isolation, maintenance and biomass preparation

Isolates OF1^T, OF3, OF8, IF11, IF17 and IF19 were recovered from an alkaline soil (pH 9.9- 10.4) adjacent to Lake Lonar (19°58'21.4"N/76°30'17.9E), a meteoric alkaline soda lake located in the Buldhana district of Maharashtra, India, using a standard dilution plate procedure [30]. Briefly, one gram of the soil was suspended in 9.0 ml of ¼ strength Ringer's solution (Oxoid), mixed on a tumble shaker for an hour prior to the preparation of serial dilutions. Aliquots (100 µl) of each serial dilution were spread over the surfaces of starch-casein agar plates [41], adjusted to pH 8.5 with 1N NaOH, and supplemented with 5% (w/v) sodium chloride and cycloheximide and nystatin (each at 50 µg ml⁻¹); the plates were dried for 15 minutes prior to inoculation, as recommended by Vickers and Williams [81]. Inoculated plates, three per dilution, were incubated at 28 °C for 4 weeks when typical streptomycete-like colonies were detected; representative isolates were subcultured onto starch-casein agar adjusted to pH 8.5 and supplemented with 5% w/v NaCl. The isolates and *S. alkaliphilus* DSM 42118^T [1], *Streptomyces calidiresistens* DSM 42108^T [25], *S. durbertensis* DSM 104538^T [86], *Streptomyces palmae* CMU-AB204^T [74] and *Streptomyces xinghaiensis* S187^T [87] were maintained on slopes of halophilic nutrient agar [6] at room temperature and as

suspensions of spores and hyphae in 20%, v/v glycerol at -80°C .

Biomass for the molecular systematic and the chemosystematic studies was prepared by growing the isolates in shake flasks of halophilic nutrient broth [6] supplemented with 5% NaCl, w/v (pH 8.5) at 150 revolutions per minute for 2 weeks at 28°C . Cells were harvested by centrifugation and washed three times in sterile distilled water; biomass for the chemotaxonomic analyses was freeze dried and that for the molecular systematic studies stored at room temperature.

Chemotaxonomic, cultural and morphological properties

Standard chromatographic methods were used to establish the chemotaxonomic profiles of the isolates. They were examined for isomers of diaminopimelic acid [73], isoprenoid quinones [22], polar lipids [53] and whole-organism sugars [33], using appropriate controls.

Cellular fatty acids extracted from isolates OF1^T and IF17 were methylated after Miller [52] with minor modifications from Kuykendall et al. [42]. The fatty acid methyl esters were separated by gas chromatography (Hewlett Packard instrument 6890 N) and the resultant peaks automatically integrated; fatty acid names and percentages were determined using the standard Microbial Identification MIDI System, version 5 [64] and the TSBA40 version of the MIDI database. The G+C mol% of the DNA of isolates OF1^T and IF17 were determined by HPLC following the procedure described by Tamaoka and Komagata [77]; purified DNA prepared, as described later, was hydrolyzed with P1 nuclease, the nucleotides dephosphorylated with bovine alkaline phosphatase [51] then analyzed by HPLC (Shimadzu Corp., Japan). Lambda- DNA and three DNAs with published genome sequences within the G+C range 43–72 mol % were used as standards

and G+C values calculated from the ratio of deoxyguanosine and deoxythymidine according to Mesbah *et al.* [51].

Cultural features were recorded following growth on media adjusted to pH 8.5, as before, and incubated at 28 °C for 3 weeks. The isolates and corresponding reference strains were grown on tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic-salts starch, glucose- asparagine, peptone-yeast extract-iron and tyrosine agar (ISP media 1–7, respectively) [69] and on modified Bennett's [36] and halophilic nutrient [6] agar. Aerial and substrate mycelial colours and those of diffusible pigments were determined by comparison against colour charts [40].

Hyphal and spore chain features of isolates OF1^T, OF3 and OF8 were detected on halophilic nutrient agar [6], adjusted to pH 8.5, after 14 days at 28 °C, using the coverslip technique of Kawato and Shinobu [39]; the same procedure was used for isolates IF11, IF17 and IF19 through in this case the strains were grown on ISP5 agar [69] at pH 8.5. Spore arrangement and spore surface ornamentation of isolates OF1^T and IF17 were established by examining gold coated dehydrated preparations with growth taken from halophilic nutrient and ISP5 agar plates, respectively, using a scanning electron microscope (Model 1430 VP, LEO Electron Microscopy Ltd, Cambridge, England) and the procedure described by O'Donnell *et al.* [56].

Box typing

BOX-PCR fingerprints from genomic DNA extracted from the isolates were generated using the BOXA1R primer [80] and experimental conditions described previously [79].

Phylogenetic analyses

Genomic DNA was extracted from each isolate and PCR mediated amplification of 16S rRNA gene sequences achieved following Golinska *et al.* [28]. PCR products were purified using PCR purification kits (Qiagen) and sequenced at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in Warsaw using an ABI 3730xl Genetic Analyzer (Applied Biosystems). The resultant data were compared with corresponding sequences of the type strains of closely related *Streptomyces* species using the EzBioCloud server [85] and aligned using Clustal W [44]. 16S rRNA gene sequence similarities were calculated using PHYDIT version 1.0 software [19] and a maximum-parsimony (MP) phylogenetic tree generated using the genome-to-genome distance calculator (GGDC) web server [49] adapted for single genes following multiple sequence alignments using MUSCLE software [26]. Similarly, a MP tree was inferred from the alignments with the Tree Analysis using New Technology (TNT) program [29] using 1000 bootstraps together with tree bisection and reconnection branch swapping and 10 random sequence replicates. A ML tree was constructed from alignments with RAxML [72] using rapid bootstrapping and the auto maximal-relative-error (MRE) bootstrapping criterion [60]. The sequences were checked for computational bias using the χ^2 test implemented in PAUP* [75].

DNA:DNA relatedness values were estimated, in duplicate, between isolate IF17 and *S. alkaliphilus* DSM 42118^T using the service provided by the DSMZ (Braunschweig, Germany). Wet biomass of each strain was disrupted using a Constant System TS 0.75 KW machine (IUL Instruments, Germany). DNA extracted from the strains using a French pressure cell (Thermo Spectronic) was purified on a hydroxyapatite column [17] and DNA:DNA hybridizations carried out after De Ley *et al.* [24], albeit using the modifications of Huss *et al.* [35], on a model Cary 100 BIO UV/VIS spectrophotometer fitted with a Peltier thermostat 6x6 multi-well changer and a temperature controller with

an *in situ* temperature probe (Varian).

Whole genome sequencing and genome analyses

Genomic DNA was extracted from biomass of isolates OF1^T, OF3, OF8 and IF17 and from *S. alkaliphilus* DSM 42118^T, *S. calidiresistens* DSM 42108^T and *S. durbertensis* DSM 104538^T following growth on ISP 2 agar (pH 8.5) for 7 days at 28 °C using the protocol provided by MicrobesNG (Birmingham, UK; <http://www.microbesng.uk>).

Briefly, genomic DNA was extracted from wet biomass of single colonies of the strains using extraction buffer (EB) with lysozyme and RNase A for 25 min at 37 °C, proteinase K added and the preparations incubated for 5 min at 65 °C. Genomic DNA preparations were purified using equal volumes of SPRI beads, resuspended in EB buffer and DNA quantified, in triplicate, using Quantit dsDNA HS assays in an Eppendorff AF2200 plate reader (Eppendorff, Germany). Genomic DNA was sequenced using a HiSeq instrument (Illumina, San Diego, USA) and the 250 bp paired end in house protocol (MicrobesNG). Genomic DNA libraries were prepared using a Nextera XT Library Prep Kit (Illumina, San Diego, USA) following the manufacturer's protocol with the following modifications: two nanograms of DNA instead of one were used as input, and the PCR elongation time was increased to 1 minute from 30 seconds (MicrobesNG). DNA quantification and library preparation were carried out on a Microlab STAR automated liquid handling system (Hamilton, Birmingham, UK) and pooled libraries quantified using the Kapa Biosystems Library Quantification Kit for Illumina on a Roche light cycler 96 qPCR machine (Roche, West Sussex, UK). Reads were trimmed using Trimmomatic 0.30 [14] and those under 250 bp discarded; contigs of each strain were assembled using Spades 3.7 software [11], annotated using Prokka 1.11 [67]

and sequence based comparisons achieved using the SEED Viewer [8]. The completeness and contamination of the draft genome sequences of the isolates and the *S. alkaliphilus*, *S. calidiresistens* and *S. durbertensis* strains were estimated using CheckM [59]. The genome sequences of the isolates and the type strains of *S. alkaliphilus*, *S. calidiresistens* and *S. durbertensis* were uploaded onto the Type (Strain) Genome Server (TYGS) [47] and compared using the MASH algorithm which allows fast approximation of intergenomic relatedness between strains [57]. A phylogenomic tree was inferred with FastME [45] from GBDP distances calculated from genome sequences, branch lengths were scaled using the GBDP distance formula d5 [49], numbers above branches are GBDP pseudo- bootstrap support values based on 100 replications.

Two-way BLAST based average nucleotide identities (ANIs) between the genomes of isolates OF1^T, OF3 and OF8, between these isolates and *S. durbertensis* DSM 42118^T and between isolate IF17 and the type strains of *S. alkaliphilus* and *S. calidiresistens* were calculated using the online resource from the K. Konstantinidis group (<http://enve-omics.gatach.edu/>; [32]). The corresponding digital DNA:DNA hybridization (dDDH) values between the genomes of the isolates and the reference strains were calculated using the genome-to-genome distance calculator, GGDC [7,49]. The genomes of the isolates and the corresponding reference strains were examined for BGCs using anti-SMASH 4.0 software [13].

Phenotypic properties

The isolates and associated reference strains were examined for a range of biochemical, degradative and physiological properties using media and methods employed in a comprehensive numerical taxonomic study on the genus *Streptomyces* [83]. The tests were

carried out, in triplicate, in 12-well plates that were inoculated using a standard inoculum in sterile distilled water that corresponded to 5 on the McFarland scale [54]; the plates were inoculated using a multipoint inoculator (Mast Uri®Dot, Mast Group Ltd., Merseyside, UK) and incubated for 3 weeks at 28 °C, apart from the temperature tests. The enzymatic activities of the strains were determined using API ZYM kits (bioMerieux), according to the manufacturer's instructions. The isolates and reference strains were also tested for their ability to grow over a range of temperature (4, 10, 15, 20, 25, 28, 30, 35, 40, 41, 42, 43, 44, 45 and 50 °C) and pH (4.0–13.0 at single unit intervals) regimes and in the presence of various concentrations of sodium chloride (0–15.0 %, w/v at one unit intervals) using ISP 2 agar as the basal medium; the pH values were achieved using KH₂PO₄/HCl (pH range 4–5), KH₂PO₄/K₂HPO₄ (0.1M both, pH range 6–8) and K₂HPO₄/NaOH (pH range 9–13) buffer systems.

Results and discussion

The isolates shared chemotaxonomic, cultural and morphological properties consistent with their classification in the genus *Streptomyces* [38,55]. They formed extensively branched substrate mycelia and aerial hyphae that differentiated into straight chains of spores. The isolates produced whole organism hydrolysates containing LL-A₂pm, glucose and xylose with either ribose (isolates OF1^T, OF3 and OF8) or mannose (isolates IF11, IF17 and IF19), contained either hexa- or octa-hydrogenated menaquinones with nine isoprene units as predominant isoprenologues and complex polar lipid patterns containing diphosphatidylglycerol, phosphatidylethanolamine (diagnostic component), phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides, as shown in **Figure S1**. It is apparent from variations in the BOX-PCR fingerprint data that neither set of

isolates are clones though isolates IF11, IF17 and IF19 have very similar fingerprints (**Fig. S2**). In addition, all of the isolates grew well on the nutrient agar media producing a range of pigments (**Table S1**). Isolates IF11, IF17 and IF19 gave identical results on all of the media generally forming reddish brown substrate mycelia and a white to grey aerial spore mass. In contrast, isolates OF1, OF3 and OF8 showed variable results on some of the media but tended to form brown or yellow substrate mycelia and a white to grey aerial spore mass. Distinctive diffusible pigments were produced on some of the ISP media. Isolates IF17 and OF1^T formed long straight chains of smooth surfaced spores, and complex fatty acid patterns, as exemplified in **Figure S3** and **Table S2**, respectively. Each of the isolates contained major proportions of anteiso-C_{15:0} (38.2 and 22.1%, respectively) and iso-C_{16:0} (20.6 and 23.1%) with iso-C_{15:0} (19%; strain IF17) and anteiso-C_{17:0} (18%; strain OF1^T), profiles consistent with their classification in the genus *Streptomyces* [38, 55]. The DNA G+C values of isolates IF17 and OF1^T were 72.4 and 72.0 mol%, respectively.

Nearly complete 16S rRNA gene sequences were obtained for isolates OF1^T, OF3 and OF8 (1534 nucleotides [nt]), the GenBank accession numbers are MH430523, MH430525 and MH430527, respectively. The isolates had identical 16S rRNA gene sequences, formed a well supported branch in the *Streptomyces* 16S rRNA gene tree with *S. durbertensis* DSM 104538^T (**Fig. 1**) and shared a 99.3% 16S rRNA gene sequence similarity with the latter, which corresponds to 10 nt differences at 1520 locations. The isolates were relatively closely related to the type strains of *S. palmae* (98.1% similarity) and *S. xinghaiensis* (98.3% similarity), values well below the 99% threshold used to assign closely related actinobacteria to the same species [49]. Nearly complete 16S rRNA gene sequences were acquired for isolates IF11, IF17 and IF19 (1389, 1535 and 1402 nt), respectively; the corresponding GenBank accession numbers are MH425397, MH425398

and MH425399. Isolates IF17 and IF19 with identical 16S rRNA gene sequences shared a 99.9% sequence similarity with isolate IF11, a value equivalent to a single nt difference at 1387 and 1385 locations, respectively. It is apparent from **Figure 1** that these isolates form a well supported clade in the *Streptomyces* 16S rRNA gene tree with the type strains of *S. alkaliphilus* and *S. calidiresistens* sharing 16S rRNA gene sequence similarities with the former, their closest phylogenetic neighbour, of either 99.6 or 99.7%; the corresponding range with the *S. calidiresistens* strain is 99.4 to 99.5%. Isolate IF17 and the type strain of *S. alkaliphilus* shared a mean DNA:DNA relatedness of $63.6 \pm 1.1\%$, a value below the 70% threshold recommended for assigning closely related strains to the same genomic species [82]. However, results such as these need to be interpreted with care as dDDH methods based on comparisons of whole genome sequences provide better quality data for assigning closely related strains to the same or different species than corresponding values derived from the application of experimental methods which are prone to experimental error (7,49,54).

The general features of the draft genome sequences of isolates IF17, OF1^T, OF3 and OF8 and those of the type strains of their closely related phylogenetic neighbours are shown in **Table S3**. The genome sizes of the strains range from 5.8 to 6.2 Mb showing that they are moderately gifted *sensu* Baltz [10]. The completeness of assemblies is high (94.8 – 97.2%) with very low levels of contamination (0.3 – 3.8 %), indicating that the draft genomes are of good quality (**Table S3**).

The dDNA G+C similarity values of isolate IF17 and *S. alkaliphilus* DSM 42118^T were 72.4 and 72.5 %, respectively; the corresponding similarity values of isolates OF1^T, OF3 and OF8 and *S. durbertensis* DSM 104538^T ranged from 72.0 to 72.4%, all of the values are within the 1 % range expected of strains belonging to the same species [50]. The

relative distribution of the different functional gene classes shown in **Figure S4** are similar to those of the type strain of *Streptomyces leeuwenhoekii* [16] and the model strains, *Streptomyces coelicolor* A3(2) [12] and *Streptomyces lividans* 66 [23].

It is evident from the phylogenomic tree (**Fig. 2**) that isolate IF17 is closely related to the type strain of *S. alkaliphilus* and less so to *S. calidiresistens* and that isolates OF1^T, OF3 and OF8 have identical or almost identical genomic sequences that distinguish them from *S. durbertensis* thereby confirming the relationships found between all of these strains in the 16S rRNA gene tree. The recommended thresholds used to distinguish between closely related prokaryotic species based on ANI and dDDH similarities are 95 to 96% [21,62] and 70% [20,21,82], respectively. It is evident from **Table 1** that on this basis isolates OF1^T, OF3 and OF8 belong to the same genomic species. The dDDH values between the genome sequences of these isolates and *S. durbertensis* DSM 10538^T, their closest phylogenomic neighbour, are low at 45.2, 45.1 and 44.9 %, respectively; the corresponding shared ANI similarity was 92.0 % indicating that the isolates and the *S. durbertensis* strain belong to different species. In contrast, the high ANI and dDDH similarities (98.0 and 79.6%, respectively) found between isolate IF17 and *S. alkaliphilus* DSM 42118^T are well above the recommended thresholds used to assign closely related strains to the same species. This latter value is in sharp contrast to the 63.6% similarity found between these organisms using the laboratory based method though, as mentioned earlier, *in silico* DDH values provide more accurate data than corresponding laboratory based procedures [50,55], as is the case with estimates of dDNA G+C composition [50,55]. Low ANI and DDH similarities clearly separate isolates IF17 and OF1^T (**Table 1**).

Excellent congruence was found between the replicated phenotypic tests carried out on the isolates and associated reference strains. Each set of isolates had many phenotypic

features in common though in each case a few properties can be weighted to distinguish
 between them (**Table S4**). All of the isolates can be separated from the type strains of
 their closest phylogenomic neighbours using a combination of phenotypic properties
 (**Tables 2 and 3**). Isolates OF1, OF 3 and OF8 can be distinguished from *S. durbertensis*
 DSM 104538^T based on pH and temperature profiles and by positive results for esterase
 (C4), esterase lipase (C8) and lipase (C14), nitrate reduction, Tween 80 degradation and
 use of D-raffinose as a sole carbon source. In contrast, the *S. durbertensis* strain, unlike the
 isolates, degraded starch, used adonitol, L-arabinose, β -methyl-D-glucosamide, D-
 glucosamine, D-glucose, glycerol, glycogen, D- lactose, D-mannitol, benzoate, butyrate,
 hippurate and propionate (sodium salts) as sole carbon sources and showed a much greater
 ability to use sole nitrogen sources. Similarly, the isolates can be separated from the type
 strains of *S. palmae* and *S. xinghaiensis* using combinations of phenotypic features.
 Phenotypic features that distinguish the reference strains can be seen in **Table 2**.
 Isolates IF11, IF17 and IF19 and *S. alkaliphilus* DSM 42118^T and *S. calidiresistens* DSM
 2108^T, their closest phylogenomic neighbours, grow from pH 8 to pH 10, indicating that
 they are alkaliphilic streptomycetes, and from 20 to 40 °C. **Table 3** shows that while the
 isolates and the type strains of their two closest relatives have many phenotypic features
 (unit characters) in common they can be distinguished using a few such properties.
 However, 96 and 79.2 % of the unit characters were shared by the isolates and by them
 and the *S. alkaliphilus* strain, respectively, values above the threshold used to assign
 closely related strains to *Streptomyces* species in numerical taxonomic studies based on
 the same experimental procedures and the simple matching coefficient, which includes
 both positive and negative similarities [83,84]; the corresponding value between the
 isolates and *S. calidiresistens* strain was 65% hence well below the species threshold,

thereby indicating that they belong to different taxospecies. In contrast, the close phylogenomic relationship between isolate IF17, *S. alkaliphilus* DSM 42118^T and *S. calidiresistens* DSM 42108^T is supported by morphological data as they all produce long chains of smooth-surfaced spores [1,25], a result which provides further evidence of a correlation between phylogeny of well separated streptomycete clades and associated phenotypic data [43].

It can be concluded from the extensive phenotypic data that isolates OF1^T, OF3 and OF8 belong to the same taxospecies and can be distinguished from the type strain of *S. durbertensis*, their closest phylogenomic neighbour, by a wealth of genomic and phenotypic data, notably by low ANI and dDDH similarities. Consequently, it is proposed that the isolates OF1^T, OF3 and OF8 be given species status as *Streptomyces alkaliterrae* sp. nov., with isolate OF1^T as the type strain. The description of the new species is given in **Table 4**.

It is also apparent that isolates IF11, IF17 and IF19 form a well supported lineage in the 16S rRNA gene tree together with the type strains of *S. alkaliphilus* and *S. calidiresistens*. The isolates are most closely related to the *S. alkaliphilus* strain and together with the latter form a well defined taxospecies. In addition, isolate IF17 and *S. alkaliphilus* DSM 42118^T form a well supported subclade in the phylogenomic tree, produce straight chains of smooth-surfaced spores on aerial hyphae, share almost identical dG+C similarity values and ANI and dDDH similarities that are well above the thresholds used to assign closely related strains to the same species. All of these datasets show that these isolates are authentic strains of *S. alkaliphilus* [1]. Further, in light of the data acquired in this study emended descriptions are given for *S. alkaliphilus* [1], *S. calidiresistens* [25] and *S. durbertensis* [86], notably on genomic sizes and dDNA G+C values, properties considered

to be phylogenetically conserved [55].

The genomes of isolates IF17, OF1^T, OF3 and OF8 and their closest phylogenomic neighbours contained between 24 (isolate OF8) and 41 (*S. alkaliphilus* DSM 42118^T) BGCs (**Fig. 3**) thereby falling within the range expected for *Streptomyces* strains [10,18]. In general, the genomes of these strains contain a similar range of bioclusters, notably ones associated with the production of siderophores, terpenes, non-ribosomal peptide and type III polyketide synthases. **Table S5** shows that many of the bioclusters detected in the genomes of the isolates and their closest phylogenomic neighbours are discontinuously distributed. Examples include bioclusters predicted to encode for novel specialised metabolites, as exemplified by a lassopeptide associated with the isolates and the *S. calidiresistens* and *S. durbertensis* strains, a linaridin associated with the *S. alkaliphilus* and *S. calidiresistens* strains, a NPRS-like biocluster considered to encode for rhizomide A/B/C compounds associated with *S. alkaliphilus* DSM 42118^T strain and NRPS bioclusters expressing for thiocoraline and virginiamycin associated with isolates OF1, OF3 and OF8, and the *S. durbertensis* strain. The discontinuous distribution of bioclusters in the three *S. alkaliterrae* isolates provide further evidence that members of the some *Streptomyces* species can display different specialised metabolites [46,58]. These results provide further evidence that alkiphilic streptomycetes are a potentially rich source of drug-like molecules [63,71].

Emended description of *Streptomyces alkaliphilus* Akhwale et al. 2015

This description is based on data taken from the current study and from the earlier description of the species [1].

Grows from pH 8 to 10, between 20-40 °C and in the presence 0-5%, w/v NaCl. Positive

for alkaline phosphatase, cystine and leucine arylamidases, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase, but not for acid phosphatase, α -fucosidase, α - or β -galactosidases, β -glucuronidase, N-acetyl- β -glucosaminidase or β -glucosidase (API-ZYM tests). Degrades carboxymethylcellulose, Tweens 40 and 60, but not adenine, casein, chitin, DNA, elastin, guanine, hypoxanthine, pectin, Tween 20, L-tyrosine, uric acid, xanthine and xylan. Aesculin is hydrolysed but not allantoin or urea. Nitrate is not reduced. Adonitol, L-arabinose, D- and L-arabitol, D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, glycogen, D-lactose, D-maltose, D-mannitol, D- ribose, L-rhamnose, D-sorbitol, sucrose, D-xylose, and acetate, benzoate, citrate, fumarate, hippurate, malonate, oxalate, propionate and pyruvate (sodium salts) are used as sole carbon sources, but not D-glucosamine, D-glucuronic acid, *meso*-inositol, *p*-hydroxy-benzoic acid or sodium adipate. L-alanine, L-arginine, L-asparagine, L-histidine, L-*isoleucine*, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine and L-valine are used as sole nitrogen sources, but not acetamide and glutamic acid. The dG+C content of the genome of the type-strain is 72.5%, its approximate size 5.76 Mbp and its GenBank accession number is VKHT000000000.

Emended description of *Streptomyces calidiresistens* Duan et al. 2014

This description is based on data taken from the current study and from the earlier description of the species [25].

Grows from pH 8 to 10, between 20-40 °C and in the presence 0-1%, w/v NaCl. Positive for alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase, but negative for acid phosphatase, α -fucosidase, α - and β -galactosidases, β -glucuronidase, N-

acetyl- β - glucosaminidase and β -glucosidase (API-ZYM tests). Degrades carboxymethylcellulose, Tweens 40, 60 and 80, but not adenine, casein, chitin, DNA, elastin, guanine, hypoxanthine, pectin, starch, Tween 20, L-tyrosine, uric acid, xanthine or xylan. Aesculin and arbutin are hydrolysed, but not allantoin or urea. Nitrate is not reduced. L-arabinose, D- and L-arabitol, D- glucosamine, glycogen, D-maltose, D-mannitol, L-rhamnose, D-sorbitol, xylitol, D-xylose, and acetate, fumarate, malonate and pyruvate (sodium salts) are used as sole carbon sources, but not adonitol, amygdalin, D-cellobiose, dextrin, *meso*-erythritol, D-fructose, D-galactose, D- glucose, α - or β -*methyl*-D-glucoside, D-glucuronic acid, glycerol, *meso*-inositol, inulin, D- lactose, D-melezitose, D-melibiose, D-raffinose, D-ribose, D-salicin, sucrose, *p-hydroxy*- benzoic acid, adipate, benzoate, butyrate, citrate, hippurate, oxalate, propionate or succinate (sodium salts). L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-histidine, L- *isoleucine*, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine and L-valine as sole nitrogen sources, but not acetamide, ethanolamine, glutamic acid or L-hydroxyproline. The dG+C of the genome of the type strain is 73%, its approximate size 6.19 Mbp and its GenBank accession number is VKHS000000000.

Emended description of *Streptomyces durbertensis* Yu et al. 2018

The description is based on data taken from this study and the earlier description of the species [86]. Grows from pH 6 to 12, between 15-42 °C and in the presence 0-7%, w/v NaCl. Positive for alkaline phosphatase, α -chymotrypsin, cystine, leucine and valine arylamidases, naphthol-AS- BI-phosphohydrolase and trypsin, but negative for acid phosphatase, esterase (C4), esterase lipase (C8), α -fucosidase, α - and β -galactosidases, N-*acetyl*- β -glucosaminidase, β -glucosidase, β -glucuronidase, lipase (C14) or α -mannosidase (API-

ZYM tests). Degrades starch and Tweens 40 and 60, but not adenine, carboxymethylcellulose, casein, chitin, DNA, elastin, guanine, hypoxanthine, pectin, Tweens 20 and 80, L-tyrosine, uric acid, xanthine or xylan. Aesculin is hydrolysed, but not allantoin, arbutin or urea. Nitrate is not reduced. Adonitol, amygdalin, L- arabinose, D- and L-arabitol, D-cellobiose, dextrin, *meso*-erythritol, D-fructose, D-galactose, D-glucose, α - and β -*methyl*-D-glucoside, D-glucosamine, glycerol, glycogen, *meso*-inositol, D- lactose, D-maltose, D-mannitol, D-melezitose, D-melibiose, D-ribose, sorbitol, D-sucrose, D-trehalose, xylitol, D-xylose, *p-hydroxy*-benzoic acid, acetate, adipate, citrate, benzoate, butyrate, fumarate, hippurate, malonate, propionate, pyruvate and succinate (sodiumsalts), but not D-glucuronic acid, inulin, L-rhamnose, D-salicin or sodium oxalate. L-Alanine, L- arginine, L-asparagine, ethanolamine, L-histidine L-hydroxyproline, L-*isoleucine*, L- methionine, L- phenylalanine, L-proline L-serine, L-treonine and L-valine are used as a sole nitrogen sources, but not acetamide, L-aspartic acid, L-cysteine or L-glutamic acid.

The dG+C content of the genome of the type strain is 72.4%, its approximate size 5.92 Mbp and its GenBank accession number is WMLF00000000.

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Conflicts of interest

The authors declare that they do not have any conflicts of interest.

Ethical statement

This article does not contain any studies with human participants or animals.

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Table 1. Average nucleotide identity (ANI) and digital DNA:DNA hybridization (dDDH) values found between representative isolates and between them and their closest phylogenetic neighbours.

Strains		ANI (%)	dDDH (%)
Isolate IF17	<i>S. alkaliphilus</i> DSM 42118 ^T	98.0	79.6
	<i>S. calidiresistens</i> DSM 2108 ^T	91.5	42.0
Isolate OF1 ^T	<i>S. durbertensis</i> DSM 10538 ^T	92.0	45.2
Isolate OF1 ^T	Isolate OF3	99.6	95.8
Isolate OF1 ^T	Isolate OF8	100	99.9
Isolate OF3	Isolate OF8	99.6	95.7
OF3	<i>S. durbertensis</i> DSM 10538 ^T	92.0	45.1
OF8	<i>S. durbertensis</i> DSM 10538 ^T	92.0	44.9
Isolate OF1 ^T	Isolate IF17	80.7	23.2
<i>S. alkaliphilus</i> DSM 42118 ^T	<i>S. calidiresistens</i> DSM 42108 ^T	91.5	42.1

Table 2. Phenotypic properties that distinguish isolates OF1^T, OF3 and OF8 from their closest phylogenetic relatives.

Characteristics	Isolates OF1 ^T , OF3 and OF8	<i>S. durbertensis</i> DSM 104538 ^T	<i>S. palmae</i> CMU-AB204 ^T	<i>S. xinghaiensis</i> S187 ^T
API-ZYM tests:				
Acid phosphatase, α -Galactosidase	-	-	+	-
α -Chymotrypsin	+	+	+	-
Esterase (C4)	+	-	+	+
Esterase lipase (C8)	+	-	+	+
Lipase (C14)	+	-	-	-
Trypsin	+	+	+	-
Hydrolysis of:				
Aesculin	+	+	+	-
Arbutin	-	-	+	-
Reduction of nitrate	+	-	+	+
Degradation of:				
Starch	-	+	+	-
Tween 20	-	-	+	+
Tween 80	+	-	+	+
Sole carbon sources:				
Adonitol, L-arabinose, β -methyl-D-glucosamide, D-glucosamine, D-mannitol, sodium benzoate, sodium hippurate, sodium propionate	-	+	-	-
L-Arabitol, meso-erythritol, α -methyl-D-glucosamide, meso-inositol, D-melezitose, D-sucrose, xylitol, D-xylose	+	+	-	-
D-Cellobiose, D-fructose, D-galactose, D-maltose, D-melibiose	+	+	+	-
D-glucose, glycerol, glycogen	-	+	+	-
Inulin	-	-	+	-
D-Lactose, sodium butyrate	-	+	-	+
D-Raffinose	+	-	-	-
D-Ribose, sodium acetate, sodium citrate, sodium fumarate	+	+	-	+
D-Salicin, sodium oxalate	-	-	-	+
Sole nitrogen sources:				
L-Arginine, L-hydroxyproline, L-isoleucine, L-phenylalanine, L-serine, L-valine	-	+	+	-
Ethanolamine	+	+	-	-
L-Threonine	-	+	+	+
Optimum growth temperature (°C)	28-30	30-35	25	25

Key: +, positive; -, negative.

The strains were positive for alkaline phosphatase, cystine arylamidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase (API-ZYM tests), degradation of

754 Tweens 40 and 60, utilisation of L-asparagine and L-histidine as a sole nitrogen sources and
755 dextrin, sodium pyruvate, sodium succinate and D-trehalose as a sole carbon sources, but were
756 negative for α -fucosidase, β -galactosidase and β -glucuronidase (API-ZYM tests), allantoin and
757 urea hydrolysis, adenine, casein, chitin, DNA, elastin, guanine, hypoxanthine, L-tyrosine, uric
758 acid, xanthine and xylan degradation, use of acetamide, L-aspartic acid, L-cysteine and L-
759 glutamic acid as a sole nitrogen sources and D-glucuronic acid as a sole of carbon source.
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Table 3. Phenotypic properties that distinguish isolates IF11, IF17 and IF19 from their closest phylogenetic relatives.

Characteristics	Isolates IF11, IF17 and IF19	<i>S. alkaliphilus</i> DSM 42118 ^T	<i>S. calidiresistens</i> DSM 42108 ^T
Hydrolysis of:			
Arbutin	+	-	+
Degradation of:			
Starch	+	-	-
Tween 80	-	+	+
Sole carbon sources:			
Adonitol, D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, D-lactose, D-ribose, D-sucrose, sodium benzoate, sodium citrate, sodium hippurate, sodium oxalate, sodium propionate	+	+	-
Amygdalin, <i>meso</i> -erythritol, α - and β -methyl-D-glucoside, <i>meso</i> -inositol, inulin, D-melezitose, D-melibiose, D-raffinose, D-salicin, sodium butyrate, sodium succinate	+	-	-
D-Glucosamine	-	-	+
Xylitol	+	-	+
Sole nitrogen sources:			
L-Aspartic acid, L-cysteine	-	+	+
Ethanolamine, L-hydroxyproline	+	-	-
NaCl % w/v tolerance	0-5	0-5	0-1

Key: +; positive, -; negative.

The strains were positive for alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase (API-ZYM tests), used L-alanine, L-arginine, L-asparagine, L-histidine, *isoleucine*, L-methionine, L-phenylalanine, L-serine, L-threonine and L-valine as sole nitrogen sources, L-arabinose, D- and L-arabitol, glycogen, D-maltose, D-mannitol, L-rhamnose, sodium acetate, sodium fumarate, sodium pyruvate and D-xylose as sole carbon sources, hydrolysed aesculin and degraded Tweens 40 or 60, but were negative for acid phosphatase, α - and β -galactosidase, β -glucuronidase, N-*acetyl*- β -glucosaminidase and β -glucosidase (API-ZYM tests), allantoin and urea hydrolysis, degradation of adenine, casein, chitin, DNA, elastin, guanine, hypoxanthine, pectin, Tween 20, L-tyrosine, uric acid, xanthine and xylan, and for use of acetamide and glutamic acid as a sole nitrogen sources, and *p*-hydroxy-benzoic acid, D-glucuronic acid and sodium adipate as sole carbon sources.

Species name	<i>Streptomyces alkaliterrae</i>
Genus name	<i>Streptomyces</i>
Specific epithet	<i>alkaliterrae</i>
Species status	sp. nov.
Species etymology	<i>Streptomyces alkaliterrae</i> (al.ka.li.ter'rae. N.L. n. alkali soda ash; L. fem. n. terra soil, earth; N.L. gen. n. <i>alkaliterrae</i> of alkaline soil)
Description of the new taxon and diagnostic traits	<p>Aerobic, Gram-positive, non-acid alcohol fast, alkalophilic actinobacteria which form an extensively branched substrate mycelium that carries aerial hyphae that differentiate into long straight chains of smooth, cylindrical spores (0.7 x 1.1 µm). Grows from 15 to 45 °C, optimally around 30°C, from pH 6.0 to 12, optimally around pH 9.0, and in the presence of 0 - 11% NaCl w/v, optimally around 4% NaCl. Hydrolyses aesculin, but not allantoin, arbutin or urea. Nitrate is reduced. Degrades starch and Tweens 40, 60 and 80, but not adenine, casein, chitin, DNA, elastin, gelatin, guanine, hypoxanthine, L-tyrosine, Tween 20, uric acid, xanthine or xylan. L-arabitol, D-cellobiose, dextrin, <i>meso</i>-erythritol, D-fructose, D-galactose, α-methyl-D-glucoside, <i>meso</i>-inositol, D-maltose, D-melezitose, D-melibiose, D-raffinose, D-ribose, D-sucrose, D-trehalose, xylitol and D-xylose are used as sole carbon sources for energy and growth, but not adonitol, L-arabinose, β-methyl-D-glucosamide, D-glucosamine, D-glucose, glucuronic acid, glycerol, glycogen, inulin, D-lactose, D-mannitol, D-salicin (all at 1%, w/v). Acetate, citrate, fumarate, pyruvate and succinate (sodium salts) are metabolised as sole carbon sources, but not adipate, benzoate, butyrate, hippurate, oxalate, propionate (sodium salts) or <i>para</i>-hydroxybenzoic acid (all at 0.1 %, w/v). L-asparagine, ethanolamine and L-histidine are used as sole nitrogen sources, but not acetamide, L-aspartic acid, L-arginine, L-cysteine, L-glutamic acid, L-hydroxyproline, L-<i>isoleucine</i>, L-phenylalanine, L-serine, L-threonine or L-valine (all at 0.1%, w/v). The cell wall peptidoglycan contained <i>LL</i>-diaminopimelic acid; either hexa- or octa-hydrogenated menaquinones with nine isoprenoid units are produced. The predominant fatty acids are <i>anteiso</i>-C_{15:0}, <i>iso</i>-C_{16:0}, <i>anteiso</i>-</p>

	C _{17:0} , the major polar lipids diphosphatidylglycerol, phosphatidylethanolamine (diagnostic marker), phosphatidylinositol and the whole cell sugars are glucose, ribose and xylose.
Country of origin	India
Region of origin	Buldhana district of Maharashtra, India
Date of isolation	1 st August 2014
Source of isolation	Alkaline soil adjacent to Lonar lake
Sampling date	31 st May 2014
Geographic location	Buldhana district of Maharashtra, India
Latitude	19°58'21.4"N
Longitude	76°30'17.9E
16S rRNA gene accession nr.	MH430523
Genome accession number [RefSeq]	VJYK00000000
Genome status	Draft genome sequence
Genome size	5,999,109 bp
GC mol%	72
Number of strain in study	3
Source of isolation of non-type strain	Alkaline soil adjacent to lake Lonar
Designation of the type strain	OF1 ^T
Strain collection numbers	PCM 3001 ^T =MCC 3841 ^T =MTCC 12906 ^T

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Legends for figures:

Fig. 1. Maximum-likelihood and maximum-parsimony trees based on nearly complete 16S rRNA gene sequences using the GTR-GAMMA method showing relationships between isolates OF1^T, OF3 and OF8 and IF11, IF17 and IF19 and between them and the type strains of closely related *Streptomyces* species. The numbers at the nodes are bootstrap support values $\geq 70\%$ for ML (left) and MP (right). Bar; 0.007 substitutions per nucleotide position. The root position of the tree was determined using *Yinghuangia aomiensis* M24DS4^T as outgroup.

Fig. 2. Distribution of functional gene classes found in the genome of isolate OF1^T (blue), *Streptomyces coelicolor* A2(3) (red), *Streptomyces leuvenhoekii* DSM 42122^T (green) and *Streptomyces lividans* 66 (yellow) generated using the RAST-SEED webserver at <https://rast.nmpdr.org/>;

Fig. 3. Phylogenomic tree showing relationships between isolates NF3 and NH11^T and type strains of their closest relatives. The numbers above the branches are GBDP pseudo-bootstrap support values $>60\%$ from 100 replications with an average branch support of 90.5 %. The tree was rooted at the midpoint [27].

Fig. 4. Biosynthetic gene clusters found in the genomes of representative isolates and those of their closest phylogenetic neighbours using AntiSMASH 4.0 software.

Fig. S1. Two dimensional thin-layer chromatography of polar lipids of isolate OF1^T stained with molybdophosphoric acid (A), molybdenum blue (Sigma) (B), ninhidrin (C) and α -

814 naphthosulfuric acid (D) for the detection of total polar lipids. Chloroform:methanol:water
815 (32.5:12.5:2.0 v/v) was used in the first direction and chloroform:acetic acid:methanol: water
816 (40:7.5:6:2 v/v) in the second direction. DPG diphosphatidylglycerol, PE
817 phosphatidylethanolamine, PG phosphatidylglycerol, PI phosphatidylinositol, PIMs
818 phosphatidylinositol mannosides.

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820 **Fig. S2.** BOX PCR fingerprint patterns of genomic DNA extracted from the isolates.

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822 **Fig. S3.** Scanning electron micrograph of isolate OF1^T showing straight chains of smooth
823 surfaced, cylindrical spores following growth on halophilic nutrient agar for 2 weeks at 28 °C.
824 Bar, 10 µm.

Fig. S1. Two dimensional thin-layer chromatography of polar lipids of isolate OF1^T stained with molybdophosphoric acid (A), molybdenum blue (Sigma) (B), ninhydrin (C) and α-naphtosulfuric acid (D) for the detection of total polar lipids. Chloroform:methanol:water (32.5:12.5:2.0 v/v) was used in the first direction and chloroform:acetic acid:methanol: water (40:7.5:6:2 v/v) in the second direction. DPG diphosphatidylglycerol, PE phosphatidylethanolamine, PG phosphatidylglycerol, PI phosphatidylinositol, PIMs phosphatidylinositol mannosides.

Fig. S2. BOX PCR fingerprint patterns of genomic DNA extracted from the isolates.

Fig. S3. Scanning electron micrograph of isolate OF1^T showing straight chains of smooth surfaced, cylindrical spores following growth on halophilic nutrient agar for 2 weeks at 28 °C. Bar, 10 μm

Table S1. Cellular fatty acid profiles of the representative isolates.

Fatty acids	OF1 ^T	IF17
C _{14:0}	0.4	0.1
C _{15:0}	2.6	0.4
C _{16:0}	4.2	0.8
C _{17:0}	0.7	-
C _{15:1} ω6c	0.3	-
C _{15:1} B	-	0.7
C _{16:1} cis 9	-	1.6
C _{17:1} ω6c	0.2	-
C _{17:1} ω8c	0.7	-
C _{16:0} 9-methyl	-	0.2
C _{17:0} 10-methyl	0.5	-
<i>anteiso</i> -C _{13:0}	0.1	-
<i>anteiso</i> - C _{15:0}	22.1	38.2
<i>anteiso</i> - C _{17:0}	18.1	5.7
<i>anteiso</i> - C _{17:1} C	-	0.2
<i>anteiso</i> -C _{17:1} ω9c	4.4	-
<i>iso</i> -C _{13:0}	0.1	0.2
<i>iso</i> -C _{14:0}	1.7	7.4
<i>iso</i> -C _{15:0}	7.2	18.9
<i>iso</i> -C _{16:0}	23.1	20.6
<i>iso</i> -C _{16:1} H	2.6	2.8
<i>iso</i> -C _{17:0}	4.9	2.1
<i>iso</i> -C _{17:1} ω9c	3.2	-
<i>iso</i> -C _{18:0}	0.4	-
<i>iso</i> -C _{18:1} H	0.6	-
Sum in feature 3	2.0	-

Summed feature 3 comprises *iso*-C_{15:0} 2OH and C_{16:1} ω7c.

Table S2. Growth and cultural properties of isolates grown on nutrient media for 3 weeks at 28 °C.

Isolates												
Media	IF11				IF17				IF19			
	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments
ISP1	+++	Light gray	Dark reddish brown	Deep brown	+++	Light gray	Dark reddish brown	Deep brown	+++	Light gray	Dark reddish brown	Deep brown
ISP2	++	White	Light orange yellow	None	++	White	Light orange yellow	None	++	White	Orange yellow	None
ISP3	++	Light gray	Moderate olive	None	++	Light gray	Moderate olive	None	++	Light gray	Moderate olive	None
ISP4	+++	Light gray	Dark brown	Moderate yellowish brown	+++	Light gray	Dark brown	Moderate yellowish brown	+++	Light gray	Dark brown	Moderate yellowish brown
ISP5	+++	Light gray	Grayish reddish brown	None	+++	Light gray	Grayish reddish brown	None	+++	Light gray	Grayish reddish brown	None
ISP6	+++	Pale purple	Dark reddish brown	None	+++	Pale purple	Dark reddish brown	None	+++	Pale purple	Dark reddish brown	None
ISP7	+++	Light gray	Deep reddish brown	None	+++	Light gray	Deep reddish brown	None	+++	Light gray	Deep reddish brown	None
HNA	+++	Yellowish white	Dark orange yellow	None	+++	Yellowish white	Dark orange yellow	None	+++	Greenish white	Dark orange yellow	None
MBA	+++	Light gray	Dark brown	None	+++	Light gray	Dark brown	None	+++	Light gray	Dark brown	None
Isolates												
Media	OF1 ^T				OF3				OF8			
	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments
ISP1	+++	White	Moderate yellow	None	+++	White	Moderate yellow	Moderate yellow	+++	White	Moderate yellow	Moderate yellow
ISP2	+++	White	Strong brown	None	+++	White	Strong brown	None	+++	White	Strong brown	None
ISP3	+++	None	Moderate yellow	Pale purple	+++	None	Moderate yellow	Pale purple	+++	None	Moderate yellow	Pale purple
ISP4	+++	White	Light grayish yellowish brown	None	+++	White	Strong yellow	None	+++	White	Light grayish yellowish brown	None
ISP5	+++	Light gray	Light brownish gray	None	+++	None	Strong reddish orange	None	+++	None	Strong reddish orange	None
ISP6	+++	Light gray	Grayish brown	Grayish yellowish brown	+++	None	Grayish brown	Grayish yellowish brown	+++	Light gray	Grayish brown	Grayish yellowish brown
ISP7	++	White	Grayish yellow		++	None	Pale yellow		++	Light gray	Light grayish brown	None
HNA	+++	White	Light greenish yellow	None	+++	White	Light greenish yellow	None	+++	White	Light greenish yellow	None
MBA	++	None	Strong reddish orange	None	++	None	Strong reddish orange	None	++	None	Strong reddish orange	None

Key: ISP1; tryptone-yeast extract agar, ISP2; yeast extract-malt extract agar, ISP3; oatmeal agar, ISP4; inorganic salts-starch agar, ISP5; glucose-asparagine agar, ISP6; peptone-yeast extract-iron, ISP7; tyrosine agar, HNA; halophilic nutrient agar, MBA; modified Bennett's agar, +++; abundant growth, ++; good growth.

Table S3. Phenotypic properties that distinguish between each set of isolates

Characteristics	Isolates		
	IF11	IF17	IF19
API-ZYM tests:			
α-Chymotrypsin	-	+	+
α-Glucosidase	-	-	+
Lipase (C14)	-	-	+
α-Mannosidase	-	-	+
Trypsin	-	+	+
Biochemical tests			
Reduction of nitrate	+	-	-
Growth on sole carbon source (1%, w/v):			
D-Trehalose	-	+	-
Tolerance tests			
pH growth range	8-10	8-10	8-11
Temperature growth range (°C)	20-43	25-43	20-43

Characteristics	Isolates		
	OF1 ^T	OF3	OF8
API-ZYM tests:			
N-acetyl-β-Glucosaminidase	+	-	-
α-Glucosidase	-	+	+
β-Glucosidase	+	+	-
α-Mannosidase	+	-	-
Growth on sole carbon sources			
(at 1%, w/v):			
Amygdalin	+	+	-
D-Arabitol	+	-	-
L-Rhamnose	+	-	-
Growth on sole nitrogen sources (at 0.1%, w/v):			
L-Alanine	-	+	-
L-Methionine	-	+	-
Tolerance tests			
pH growth range	6-12	6-11	6-11
Temperature growth range (°C)	15-45	15-45	15-50
NaCl tolerance (% w/v)	11	8	10

Key: +, positive; -, negative.

Table S4. General features of the genomes of the representative isolates and their closest phylogenetic neighbours

Properties	Isolate IF17	<i>S. alkaliphilus</i> DSM 42118 ^T	<i>S. calidiresistens</i> DSM 42108 ^T	Isolate OF1 ^T	Isolate OF3	Isolate OF8	<i>S. durbertensis</i> DSM 104538 ^T
Genome size	5870448	5761373	6196881	5999109	5977613	6036367	5923645
Contig number	1861	1801	1643	658	748	473	1002
Digital DNA G+C content (%)	72.4	72.5	73.0	72.0	72.0	72.0	72.4
L50	308	328	280	120	111	67	165
N50	5488	5021	6206	15903	15600	26709	10866
Number of reads	4478765	1021815	2270261	2349987	4148352	2850634	406599
Mean coverage	249.0	70.2	121.7	170.0	342.0	229.0	30.0
Genes (total)	5087	5184	5572	5616	5683	5559	5612
CDSs (total)	5019	5116	5502	5539	5601	5472	5528
CDSs (with protein)	4762	4940	5271	5334	5463	5342	5395
CDSs (without protein)	257	176	231	205	138	130	133
RNA genes	68	68	70	77	82	87	84
rRNAs (5S, 16S, 23S)	5, 1, 3	4, 1, 3	5, 1, 3	6, 1, 6	6, 3, 9	6, 3, 9	7, 1, 12
Complete rRNAs (5S, 16S, 23S)	5, 1, 1	4, 1, 1	5, 1, 1	6, 1, 1	4, 1, 0	4, 1, 0	4, 1, 0
Partial rRNAs (5S, 16S, 23S)	0, 0, 2	0, 0, 2	0, 0, 2	0, 0, 5	2, 2, 9	2, 2, 9	3, 0, 12
tRNAs	56	57	58	61	61	66	61
GenBank accession number	VJYJ01000000	VKHT00000000	VKHS00000000	VJYK00000000	JABJWZ00000000	JABJXA00000000	WMLF00000000