The present study was designed to establish the taxonomic relationship between pH 5.0 and 5.5, i.e. they are neutrotolerant. Members of the genus Streptomyces are able to grow from pH 4.5 to 7.5 with optimal growth at pH 6.5. Strain BK168T was isolated from a plate of starch-casein agar (Küster & Williams, 1964), supplemented with cycloheximide and nystatin (each at 25 μg ml⁻¹) and incubated at 28 °C for 21 days. The organism had chemical and morphological features consistent with its classification in the genus Streptomyces. 16S rRNA gene sequence analysis supported classification of the strain in the genus Streptomyces and showed that it formed a distinct phyletic line loosely associated with members of the Streptomyces yeochonensis clade. It was related most closely to Streptomyces paucisporeus 1413T (98.6 %16S rRNA gene sequence similarity), but could be distinguished from the latter based on the low level of DNA–DNA relatedness (40 %). It was readily distinguished from the type strains of all species assigned to the S. yeochonensis clade based on a combination of phenotypic properties. Strain BK168T (=KACC 20908T=NCIMB 14704T) should therefore be classified as the type strain of a novel species of the genus Streptomyces, for which the name Streptomyces cocklensis sp. nov. is proposed. The organism produces the antibiotic dioxamycin.

The taxonomic position of a streptomycete isolated from soil collected from Cockle Park Experimental Farm, Northumberland, UK, was determined by using a polyphasic approach. The organism had chemical and morphological features consistent with its classification in the genus Streptomyces. 16S rRNA gene sequence analysis supported classification of the strain in the genus Streptomyces and showed that it formed a distinct phyletic line loosely associated with members of the Streptomyces yeochonensis clade. It was related most closely to Streptomyces paucisporeus 1413T (98.6 %16S rRNA gene sequence similarity), but could be distinguished from the latter based on the low level of DNA–DNA relatedness (40 %). It was readily distinguished from the type strains of all species assigned to the S. yeochonensis clade based on a combination of phenotypic properties. Strain BK168T (=KACC 20908T=NCIMB 14704T) should therefore be classified as the type strain of a novel species of the genus Streptomyces, for which the name Streptomyces cocklensis sp. nov. is proposed. The organism produces the antibiotic dioxamycin.

Members of the genus Streptomyces remain a rich source of novel bioactive compounds, notably antibiotics (Bérdy, 2005; Goodfellow & Fiedler, 2010). Another unique feature of the genus is the large number of species it contains, almost 600 at the time of writing (http://www.bacterio.cict.fr/s/streptomycesa.html). Relationships within this taxonomically complex genus have been clarified by the application of genotypic and phenotypic procedures (Manfio et al., 1995; Anderson & Wellington, 2001; Lanoot et al., 2004, 2005a) so that it is now apparent that many type strains can be assigned to distinct multi-membered 16S rRNA gene clades (Lanoot et al., 2005b; Liu et al., 2005; Xu et al., 2006; Goodfellow et al., 2007; Quintana et al., 2008; Kumar & Goodfellow, 2010; Rong & Huang, 2010), including the Streptomyces yeonchonensis clade (Kim et al., 2004; Xu et al., 2006). Members of this taxon are able to grow from pH 4.5 to 7.5 with optimal growth between pH 5.0 and 5.5, i.e. they are neutrotolerant acidophilic streptomycetes.

The present study was designed to establish the taxonomic status of a Streptomyces strain that was isolated from an acid soil and found to grow from pH 4.5 to 10.0. It is apparent from the results of the present study that this strain, designated BK168T, merits recognition as a representative of a novel species of the genus Streptomyces. The novel species is loosely associated with the S. yeonchonensis 16S rRNA gene clade.

Strain BK168T was isolated from a plate of starch-casein agar (Küster & Williams, 1964), supplemented with cycloheximide and nystatin (each at 25 μg ml⁻¹), which had been inoculated with a pre-heated soil suspension (55 °C for 20 min) and incubated at 28 °C for 21 days. The soil sample had been collected from Palace Leas meadow hay plot 6 (Atalan et al., 2000) at Cockle Park Experimental Farm, Northumberland, UK (national grid reference NZ 209013). The organism was maintained on oatmeal agar slopes [International Streptomyces Project (ISP) medium 3; Shirling & Gottlieb, 1966] at 4 °C and as a mixture of mycelial fragments and spores in 20 % (v/v) glycerol at −20 °C. Biomass for chemotaxonomic and molecular systematic studies was grown in shake flasks of tryptone-yeast extract broth (Shirling & Gottlieb, 1966) for 7 days at 28 °C, harvested by centrifugation and washed twice in distilled water; cells for chemical studies were freeze-dried.

Genomic DNA was extracted from the isolate and PCR amplification and 16S rRNA gene sequencing were achieved as described previously (Kim et al., 1996). The
resultant, almost-complete 16S rRNA gene sequence of strain BK168<sup>T</sup> was aligned manually against corresponding sequences of representatives of the genus *Streptomyces* by using the software MEGA4 (Tamura et al., 2007). Phylogenetic trees were inferred by using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms drawn from the MEGA4 (Tamura et al., 2007) and PHYLML (Guindon & Gascuel, 2003) packages. The Jukes & Cantor (1969) model was used to generate evolutionary distance matrices for the neighbour-joining algorithm. Topologies of the resultant trees were evaluated by bootstrap analysis (Felsenstein, 1985) of the maximum-likelihood method based upon 1000 replicates by using MEGA4. *Streptomyces macrosporus* DSM 41449<sup>T</sup> (GenBank accession no. Z68099), *Streptomyces megasporus* DSM 41476<sup>T</sup> (Z68100) and *Streptomyces scabrisporus* KM-4927<sup>T</sup> (AB030585) were used as the outgroup.

It can be seen from Fig. 1 that strain BK168<sup>T</sup> forms a distinct phyletic line that is loosely associated with the *S. yeochonensis* 16S rRNA gene clade in the maximum-likelihood and maximum-parsimony analyses. However, this relationship was not supported by high bootstrap values or by neighbour-joining analysis, in which the organism was recovered in the *S. yeochonensis* 16S rRNA gene clade. The isolate was most closely related to the type strain of *Streptomyces paucisporeus*; the two strains shared 98.6 % 16S rRNA gene sequence similarity, a value which corresponded to 20 nt differences at 1401 locations. Levels of 16S rRNA gene sequence similarity between strain BK168<sup>T</sup> and the type strains of other members of the clade were 98.4 % with *Streptomyces yanglinensis* and *S. yeochonensis*, 98.3 % with *Streptomyces rubidus* and 97.6 % with *Streptomyces guanduensis*.

The level of DNA–DNA relatedness between the novel isolate and the type strain of *S. paucisporeus*, its nearest phylogenetic neighbour, was determined by using the nitrocellulose membrane filter hybridization procedure described by Seldin & Dubnau (1985). DNA probes were labelled using the non-radioactive digoxigenin High Prime System (Roche), hybridized DNA was visualized by using a digoxigenin luminescent detection kit (Roche) and the level of DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad). The two strains shared 40 % DNA–DNA relatedness, a value well below the 70 % cut-off recommended for the assignment of strains to the same genomic species (Wayne et al., 1987).

Isolate BK168<sup>T</sup> was examined for chemical markers that are characteristic of *Streptomyces* strains. Standard procedures were used to determine the diaminopimelic acid isomers (Hasegawa et al., 1983), predominant menaquinones (Collins, 1985), muramic acid type (Uchida et al., 1999) and diagnostic whole-cell sugars (Hasegawa et al., 1983), by using appropriate controls. Fatty acids were extracted, methylated and analysed by GC with the standard Sherlock Microbial Identification system (MIDI, 1999). The DNA G+C content of the isolate was determined by using the procedures described by Gonzalez & Saiz-Jimenez (2002).

Strain BK168<sup>T</sup> contained major amounts of LL-diaminopimelic acid and N-acetylated muramic acid in the cell-wall peptidoglycan, lacked diagnostic sugars in whole-organism...
hydrolysates (wall chemotype I sensu Lechevalier & Lechevalier, 1970), and contained hexa-, octa- and tetra-
hydrogenated menaquinones with nine isoprene units
[MK-9(H6, H8, H4)] as predominant isoprenologues in a
ratio of 3 : 3 : 2, respectively, and minor amounts of MK-
9(H6). The cellular fatty acid profile consisted of major
amounts of anteiso-C15:0 (27.8 %), C16:0 (23.8 %) and iso-
C16:0 (19.4 %), lesser amounts (<10 %) of anteiso-C17:0
(9.5 %), iso-C15:0 (7.4 %) and iso-C14:0 (5.8 %), and trace
amounts of iso-C17:0 and C14:0. The DNA G+C content
of strain BK168T was 73.2 mol%. All of these properties are
in line with the classification of the isolate in the genus
Streptomyces (Williams et al., 1989; Manfio et al., 1995;
Anderson & Wellington, 2001).

The isolate was examined for cultural and morphological
features after growth on several standard media at 28 °C for
3 weeks. Cultural properties were investigated by using
glucose-yeast extract-malt extract (DSMZ medium 65) and
modified Bennett’s (Jones, 1949) agars and on tryptone-
ysseum (ISP medium 1), yeast extract-malt extract (ISP
medium 2), oatmeal (ISP medium 3), inorganic salts-starch
(ISP medium 4), glycerol-asparagine (ISP medium 5) and
strains of phylogenetically closely related Streptomyces
species

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tr>
<td>Appearance on oatmeal agar</td>
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<tr>
<td>Aerial hyphae</td>
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<td>Aerial spore-mass colour</td>
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<td>Melanin production on tyrosine agar</td>
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<td>Degradation of (% w/v)</td>
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<tr>
<td>Aesculin (0.1)</td>
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<td>Casein (1.0)</td>
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<td>−</td>
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<tr>
<td>Hypoxanthine (0.4)</td>
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<td>+</td>
<td>−</td>
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<tr>
<td>Xylan (0.4)</td>
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<td>−</td>
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<td>Growth on sole carbon sources (at 1%, w/v)</td>
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<td>Trehalose</td>
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<tr>
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<td>Growth at:</td>
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<td>pH 4.5</td>
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</table>
Aliquots of the extracts were analysed by reversed-phase HPLC and diode array monitoring according to a standard protocol (Fiedler, 1993). The dominant peak in the HPLC chromatogram of the extracts was identified by HPLC–diode array detection–electrospray ionization–MS analysis as dioxamycin based on its characteristic UV-VIS profile and molecular mass of 736.3 Da. Dioxamycin, a benz[a]anthraquinone antibiotic, was first isolated from Streptomyces sp. MH406-SF1, which is closely related to Streptomyces xantholiticus (Sawa et al., 1991).

Strain BK168T and the type strains of S. guaduensis, S. paucisporus, S. rubidus, S. yanglinensis and S. yeochonensis were examined for a range of phenotypic properties by using the media and methods described by Williams et al. (1983). Strain BK168T could be distinguished from the above taxa, including the type strain of its nearest neighbour, S. paucisporus, based on a combination of phenotypic properties, notably by its ability to degrade xylan and failure to grow on L-arabinose, L-rhamnose, L-sorbose or trehalose as sole carbon sources for energy and growth (Table 1). Additional phenotypic properties are given in the species description below.

It is evident from the genotypic and phenotypic data presented that strain BK168T can be distinguished from the type strains of species classified in the S. yeochonensis 16S rRNA gene clade. We therefore propose that strain BK168T represents a novel species of the genus Streptomyces, for which the name Streptomyces cocklensis sp. nov. is proposed.

**Description of Streptomyces cocklensis sp. nov.**

*Streptomyces cocklensis* (cock.len’sis. N.L. masc. adj. cocklensis of or belonging to Cockle, referring to Cockle Park Experimental Farm, the source of the type strain).

Aerobic, Gram-positive, non-acid–alcohol-fast actinomycete that forms an extensively branched substrate mycelium which carries aerial hyphae that differentiate into short, straight chains of smooth-surfaced spores (0.9–1.0 × 1.5–1.7 μm) on oatmeal agar. Grows at 10–30 °C and at pH 4.0–10.0, but not in the presence of 3.0 % (w/v) NaCl. Hydrolyses elastin and uric acid, but not arbutin, chitin, pectin or starch. Cellobiose, D-glucose and maltose are used as sole carbon sources for energy and growth, but not D-galactose, D-mannose, L-arabinol, melibiose, D-salicyl, D-sorbitol or D-xylitol (at 1 %, w/v). Susceptible to (μg mL⁻¹) cephaleoridine hydrochloride (2), streptomycin sulphate (4) and vancomycin hydrochloride (2), but not to ampicillin (4), ciprofloxacin (2), kanamycin sulphate (8), penicillin G (2 IU mL⁻¹), rifampicin (15) or tetracycline hydrochloride (8). Additional properties are cited in the text or in Table 1. Chemotaxonomic properties are typical of the genus Streptomyces.

The type and only strain, BK168T (=KACC 20908T =NCIMB 14704T), was isolated from hay meadow plot 6 soil at Cockle Park Experimental Farm, Northumberland, UK. The species description is based on a single strain and hence serves as a description of the type strain. The DNA G+C content of the type strain is 73.2 mol%.

**Acknowledgements**

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**References**


Lanoo, B., Vancanneyt, M., Van Schoor, A., Liu, Z. & Swings, J. (2005b). Reclassification of Streptomyces nigrifaciens as a later synonym of Streptomyces flavovirens; Streptomyces citreofluorescens, Streptomyces chrysomallus subsp. chrysomallus and Streptomyces flavescens as later synonyms of Streptomyces anulatus; Streptomyces chibaensis as a later synonym of Streptomyces corchorusii; Streptomyces flaviscleroticus as a later synonym of Streptomyces minutiscleroticus; and Streptomyces lipmani, Streptomyces griseus subsp. alpha, Streptomyces griseus subsp. crotosus and Streptomyces willmorei as later synonyms of Streptomyces microflavus. Int J Syst Evol Microbiol 55, 729–731.


