Taxonomic study of neutrotolerant acidophilic actinomycetes isolated from soil and description of *Streptomyces yeochonensis* sp. nov.

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Acidophilic actinomycete strains that represent the two major neutrotolerant clusters defined by numerical taxonomy (Seong, 1992) were the subject of a polyphasic taxonomic study. The centrotype of each cluster, designated as strain JL164 (=KCTC 9924) of cluster 21 and strain CN732T (=KCTC 9926T =IMSNU 50114T =NRRL B-24245T) of cluster 13, were assigned initially to the genus *Streptomyces* on the basis of morphological and chemotaxonomic characteristics; this assignation was confirmed by 16S rRNA gene sequence data. Strain CN732T formed a distinct phyletic line within the *Streptomyces* tree, whereas strain JL164 was related closely to the type strain of *Streptomyces mirabilis*. It is evident from the present and previous studies that neutrotolerant acidophilic actinomycetes comprise taxonomically diverse groups within the variation encompassed by the genus *Streptomyces*. It is also apparent that strain CN732T and other members of cluster 13 merit recognition as a novel species, for which the name *Streptomyces yeochonensis* sp. nov. is proposed.
relatively little attention, although there is evidence from chemotaxonomic and morphological studies that they should be assigned to the genus *Streptomyces*. The aim of the present study was to determine the relationships of some neutrotolerant acidophilic actinomycetes by using a polyphasic approach.

Two major neutrotolerant acidophilic taxa were defined by Seong (1992) in a numerical phenetic study: namely, clusters 13 (five members) and 21 (nine members). Cluster 13, represented by the centrotype strain CN732\(^T\) (KCTC 9926\(^T\)=IMSNU 50114\(^T\)=NRRL B-24245\(^T\)), contained organisms that were isolated from different horizons of soil from a *Pinus thunbergii* forest, Dolsan Island, Yeochon, Republic of Korea, and cluster 21, represented by the centrotype strain JL164 (KCTC 9924), accommodated strains that were isolated from different horizons of podsol soil at Hamsterley Forest, County Durham, UK (National Grid reference NY08337). The tested strains were maintained on acidified MBA plates (Lonsdale, 1985; Seong, 1992).

Biochemical and physiological properties of the strains were examined by using procedures described by Seong (1992). Biomass for chemotaxonomic and molecular studies was prepared as described previously (Kim et al., 2003). DNA extraction, PCR and sequencing of the 16S rRNA genes of the two representative strains were also carried out by using established methods (Kim et al., 1998). The resultant sequence data were aligned with those of members of representative *Streptomyces* species by using CLUSTAL X version 3.1 (Thompson et al., 1997) and the alignment was checked manually. Evolutionary trees were constructed by using the least-squares (Fitch & Margoliash, 1967), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) algorithms and phylogenetic distances were calculated after Jukes & Cantor (1969). The topology of the phylogenetic trees was tested by bootstrap analysis (Felsenstein, 1985) of the neighbour-joining data, using the SEQBOOT and CONSENSE programs from the PHYLIP suite (Felsenstein, 1993).

The tested strains formed extensively branched substrate mycelia, chains of arthrospores that were borne on the tips of aerial hyphae and a grey aerial spore-mass. Strain JL164 formed spiral chains of spores and strain CN732\(^T\) formed straight to flexuous spore-chains. Each of the organisms contained major amounts of L-l-diaminopimelic acid in whole-organism hydrolysates, and either hexa- or octa-hydrogenated menaquinones with nine isoprene units [MK-9(H)_\text{iso}-9(H)\text{_{9}}] as predominant isoprenologues. Chemotaxonomic and morphological properties of the strains were consistent with their classification in the genus *Streptomyces* (Williams et al., 1989; Manfio et al., 1995). This conclusion is in good agreement with an earlier study, where acidophilic and neutrotolerant actinomycetes clustered together with other streptomycetes on the basis of 5S rRNA sequence data (Park et al., 1991).

Strain JL164 is related closely to the type strain of *Streptomyces mirabilis*. The two organisms share 99·6 % 16S rRNA gene sequence similarity, a value that corresponds to five nucleotide differences. Strain JL164 also showed a relatively close affinity with *Streptomyces griseoconglomerates* DSM 40499\(^\text{T}\), *Streptomyces pseudovenezuelae* NRRL ISP 5212\(^\text{T}\) and *Streptomyces resistomycticus* DSM 40133\(^\text{T}\); it shared 98·1 % 16S rRNA gene sequence similarity with these isolates, a value that equates to 26 nucleotide differences. The relationship between strain JL164 and *S. mirabilis* ATCC 27447\(^\text{T}\) was underpinned by the results from all of the treeing algorithms and by a high bootstrap value (Fig. 1). The close relationship between the two organisms was also supported by morphological and physiological properties, notably by the fact that each of the strains produces a grey aerial spore-mass, spiral chains of spores and brown soluble pigments (Shirling & Gottlieb, 1972; Lonsdale, 1985; Seong, 1992). *S. mirabilis* ATCC 27447\(^\text{T}\) has been reported not to grow at pH 4·3 (Williams et al., 1983), but this observation must be checked, as acidophilic strains tend to grow after a relatively long lag period or even fail to grow on agar plates when glycerol stocks are used as inocula.

Strain CN732\(^\text{T}\) was related most closely to *Streptomyces griseoconglomerates* DSM 40004\(^\text{T}\) (97·2 %, 39 nucleotide differences) and *Streptomyces malaysiensis* ATB-11\(^\text{T}\) (97·1 %, 40 nucleotide differences) on the basis of 16S rRNA gene sequence data. However, it is clear from Fig. 1 that this organism forms an independent phyletic line in the *Streptomyces* 16S rRNA gene tree. A 16S rRNA gene similarity value of 97·1 % between strain CN732\(^\text{T}\) and *S. griseoconglomerates* DSM 40004\(^\text{T}\), the most closely related organism, is very low when compared to the mean intrageneric similarity value of 97 % that is found between representatives of *Streptomyces* species; also, the relationship between these two organisms is not supported by a high bootstrap value. The two organisms can also be distinguished readily on physiological properties, notably by the fact that each of the strains produces a grey aerial spore-mass, spiral chains of spores and brown soluble pigments (Shirling & Gottlieb, 1972; Lonsdale, 1985; Seong, 1992). *S. mirabilis* ATCC 27447\(^\text{T}\) has been reported not to grow at pH 4·3 (Williams et al., 1983), but this observation must be checked, as acidophilic strains tend to grow after a relatively long lag period or even fail to grow on agar plates when glycerol stocks are used as inocula.
**Description of Streptomyces yeochonensis sp. nov.**

*S. yeochonensis* (ye.o.chon.en.sis. N.L. masc. adj. yeochonensis of Yeochon, a province in Korea, referring to the place where the organism was first isolated).

The description is based on the present and previous studies (Lonsdale, 1985; Seong, 1992). Aerobic, Gram-positive, non-motile, neutrotolerant acidophilic actinomycete that forms extensively branched substrate and aerial mycelia. Smooth-surfaced spores are borne in flexuous spore-chains. Aerial spore-mass colour is grey. Substrate mycelia have no distinctive colour; diffusible pigments are not produced. pH range for growth is 4.3–7.3. Casein, gelatin, guanine, starch and Tween 80 are degraded, but elastin, hypoxanthine, testosterone, Tween 20, tyrosine and xanthine are not. Good growth occurs between 25 and 37°C, but not at 12 or 45°C. The sugars erythritol, inulin, melezitose, salicin, ribitol and sorbitol (all at 1%, w/v) are used as sole carbon sources, as are \( \alpha \)-hydroxybutyric acid, D-gluconic acid, hippuric acid, \( \alpha \)-ketoglutaric acid, 2-keto-D-glucuronic acid, lactic acid, malic acid, malonic acid, oxalic acid, pyruvic acid and succinic acid (as sodium salts), albeit at 0.1%, w/v.

The type strain, CN732\(^T\) (=KCTC 9926\(^T\) =KCTC 9926\(^T\) =KCTC 9926\(^T\) =NRRL B-24245\(^T\)), was isolated from acidic soil collected in the Yeochon area of the Republic of Korea.

The results of this and earlier studies (Lonsdale, 1985; Park *et al*., 1991; Seong, 1992) indicate that neutrotolerant sporactinomycetes form a heterogeneous group of actinomycetes that belong to the genus *Streptomyces*, thereby underpinning the physiological diversity encompassed by this taxon (Williams *et al*., 1989). It is also evident that neutrotolerant streptomycetes can be distinguished readily from their acidophilic counterparts, which are classified in the genus *Streptacidiphilus* (Kim *et al*., 2003). It is clear that further comparative taxonomic studies are needed to formally describe the range of taxonomic variation encompassed by neutrotolerant streptomycetes.

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**References**


