# 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 centrotypes of each cluster, designated as strain JL164 (=KCTC 9924) of cluster 21 and strain CN732$^T$ (=KCTC 9926$^T$=IMSNU 50114$^T$=NRRL B-24245$^T$) 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 CN732$^T$ 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 CN732$^T$ and other members of cluster 13 merit recognition as a novel species, for which the name *Streptomyces yeochonensis* sp. nov. is proposed.

Acidophilic actinomycetes are common in terrestrial habitats such as acidic forest and mine drainage soils, where they are a major constituent of the actinomycete community (Williams *et al.*, 1971; Khan & Williams, 1975; Hagedorn, 1976; Lonsdale, 1985; Seong, 1992). Jensen (1928) described '*Actinomyces* (*Streptomyces*) *acidophilus*', a taxon that harboured four acidophilic actinomycetes isolated from acidic soil; apart from this, no attempt has been made to describe such organisms formally, although representative acidophilic and neutrotolerant sporoactinomycetes have been compared with neutrophilic streptomycetes in a 5S rRNA gene analysis (Park *et al.*, 1991). It has been shown that acidophilic actinomycetes consistently form two distinct aggregate taxa (namely, the neutrotolerant acidophilic and strictly acidophilic cluster-groups) on the basis of numerical phenetic data; members of the two groups share common morphological and chemotaxonomic properties (Khan & Williams, 1975; Lonsdale, 1985; Seong, 1992).

Published online ahead of print on 1 August 2003 as DOI 10.1099/ijs.0.02519-0.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains JL164 and CN732$^T$ are AF101414 and AF101415, respectively.

Members of each of the aggregate groups produce extensively branched vegetative mycelia and long chains of arthrospores on the tips of aerial hyphae. Whole-organism hydrolysates of representative strains have been shown to contain major amounts of LL-diaminopimelic acid, tetra-, hexa- and octahydrogenated menaquinones with nine isoprene units and type II phospholipids *sensu* Lechevalier *et al.* (1977). In contrast, members of the two groups show different pH ranges for growth and can be distinguished by phenotypic criteria, such as carbon utilization patterns. Neutrotolerant acidophilic actinomycetes grow at a pH range from 3·5 to around neutral, with optimal growth between pH 5·0 and 5·5 (Lonsdale, 1985); strictly acidophilic strains show a restricted pH range for growth, with optimal pH around 4·5. Members of the neutrotolerant group are less able to use a range of carbon sources than their acidophilic counterparts (Seong *et al.*, 1993, 1995).

It has been shown that some members of the strictly acidophilic group form a distinct taxon, the genus *Streptacidiphilus*, which has been assigned to the revised family *Streptomycetaceae*, together with the genera *Kitasatospora* and *Streptomyces* (Kim *et al.*, 2003). In contrast, members of the neutrotolerant acidophilic group have received

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 LL-diaminopimelic acid in whole-organism hydrolysates, and either hexa- or octahydrogenated menaquinones with nine isoprene units [MK-9(H)$_6$–9(H)$_8$] 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 griseochromogenes* DSM 40499$^T$, *Streptomyces pseudovenezuelae* NRRL-ISP 5212$^T$ and *Streptomyces resistomycificus* DSM 40133$^T$; 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$^T$ 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$^T$ 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$^T$ was related most closely to *Streptomyces griseocarneus* DSM 40004$^T$ (97·2 %, 39 nucleotide differences) and *Streptomyces malaysiensis* ATB-11$^T$ (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$^T$ and *S. griseocarneus* DSM 40004$^T$, 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 the basis of morphological properties: *S. griseocarneus* DSM 40004$^T$ forms a reddish aerial spore-mass and produces spores in verticils. In a comparison of 16S rRNA gene sequences using a BLAST search (Altschul *et al.*, 1990), strain CN732$^T$ was again found to be associated most closely with the type strain of *S. griseocarneus*, thereby confirming that there was no closer neighbour for which a 16S rRNA gene sequence is available in public databases. It is also interesting that strain CN732$^T$ shares 184/229 unit characters in common with the other members of cluster 13, namely strains CN718 (=IMSNU 50115), CN725 (=IMSNU 50128), CN727 (=IMSNU 50153) and CN731 (=IMSNU 50140), with mean similarity of 91 % (Seong, 1992).

It is evident from this and earlier studies (Lonsdale, 1985; Seong, 1992) that strain CN732$^T$ and other members of cluster 13 form a novel species within the genus *Streptomyces*, for which the name *Streptomyces yeochonensis* sp. nov. is proposed.

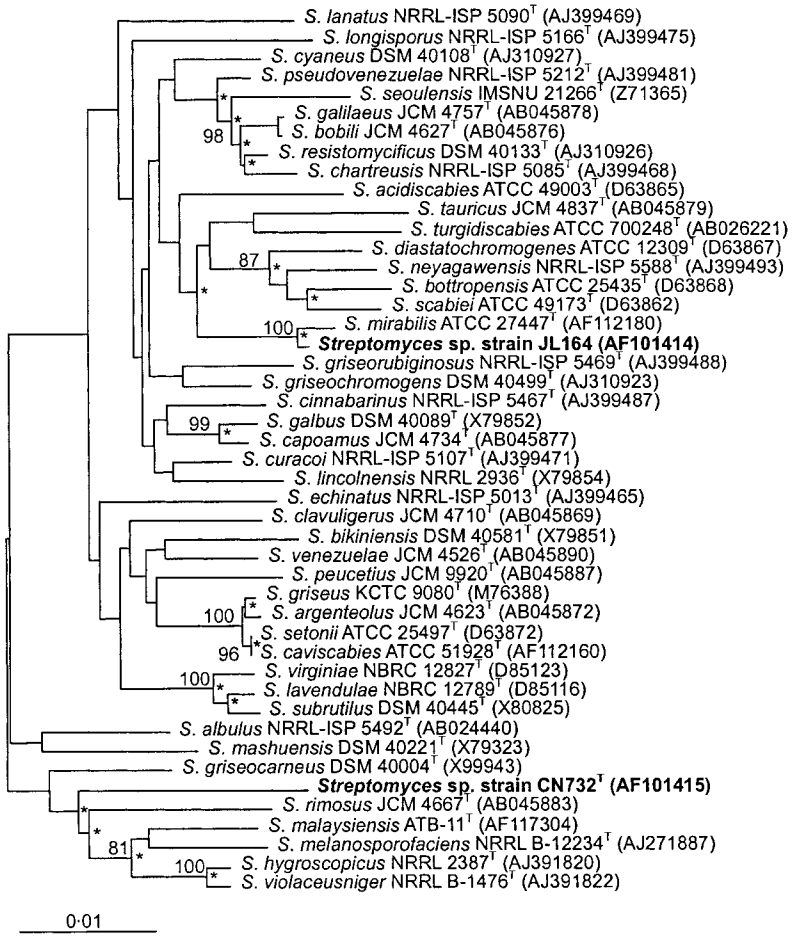


**Fig. 1.** Neighbour-joining tree based on almost-complete 16S rRNA gene sequences, showing relationships of strains JL164 and CN732$^T$ with representative species of the genus *Streptomyces*. Numbers at nodes indicate bootstrap values (%) based on 1000 resamplings; only values $>70$% are shown. Asterisks indicate branches that were also recovered by the least-squares and maximum-parsimony algorithms. Bar, 0·01 substitutions per nucleotide position.

## Description of *Streptomyces yeochonensis* sp. nov.

*Streptomyces 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 streptomycete 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 $\beta$-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$$=$IMSNU 50114$^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 sporoactinomycetes 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.

## Acknowledgements

This work was partly supported by KCTC grant KBM1000212. S. B. K. was supported by a Chevening-MOST fellowship awarded by the British Council (Seoul, Korea).

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