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NOTE

Kineococcus radiotolerans sp. nov., a radiation-resistant, Gram-positive bacterium

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A Gram-type positive, motile, coccus-shaped organism was isolated from a radioactive work area. Strain SRS30216^T is an orange-pigmented bacterium that is catalase-positive, oxidase-negative and urease-negative. The orange pigment is most likely a carotenoid with absorption peaks at approximately 444, 471 and 501 nm. Cells normally grew in clusters, but individual, motile, flagellated cells were also observed. Growth of strain SRS30216^T occurred at temperatures between 11 and 41 °C, between pH 5 and 9 and at NaCl concentrations up to and including 5%. Fatty acid composition was limited, with >90% of the fatty acids being anteiso 15:0. Alkenes of 19–24 carbons in length were detected during examination of the neutral lipids. Strain SRS30216^T demonstrated high levels of resistance to γ-radiation and desiccation. The most closely related recognized species is *Kineococcus aurantiacus* RA 333^T, which is 93% similar in 16S rDNA sequence. DNA–DNA hybridization revealed only 31% similarity between these two organisms. It is proposed that SRS30216^T (= ATCC BAA-149^T = DSM 14245^T) represents the type strain of a novel species in the genus *Kineococcus*, *Kineococcus radiotolerans* sp. nov.

Keywords: *Kineococcus*, radiation resistance, desiccation resistance

The possibility was investigated that bacteria grow in a radioactive environment at the Savannah River Site in Aiken, South Carolina, USA. At the Savannah River Site, high-level radioactive waste is stored in basins where the γ-radiation dose can be as high as 100 Gy h^{-1} (1 Gy = 100 rads). A bacterial isolate, strain SRS30126^T, was isolated from a shielded cell facility in the Savannah River Technology Center at the Savannah River Site. The radiation levels in the work area fluctuate between 0·18 Gy h^{-1} and more than 3·5 Gy h^{-1}. All isolation procedures were performed inside shielded cells and carried out using mechanical manipulators. A plastic-lined, paper-wrapped swab was moved into the shielded cell, opened using the remote manipulators inside the cell and used to wipe the metal surface on the floor of the work area. The entire swab was then placed in 10 ml PTYG nutrient solution [1% (w/v) glucose, 0·5% (w/v) yeast extract, 0·5% (w/v) tryptone, 0·5% (w/v) peptone, 0·006% (w/v) $MgSO_4.7H_2O$, 0·0006% (w/v) $CaCl_2$, pH 10·7] contained in a 15 ml centrifuge tube. The alkaline pH was chosen due to the alkaline nature of the radioactive samples normally processed in this area. The sample was stored vertically without agitation for 145 days and then used to inoculate BIOLOG GN plates. After 29 days, the BIOLOG plate containing strain SRS30126^T had four positive wells corresponding to L-arabinose, D-arabitol, cellobiose and D-serine. When the solutions from these wells were plated on PTYG medium (pH 7·2), an orange-pigmented microorganism was isolated and designated strain SRS30216^T.

Phylogenetic analysis

Genomic DNA from strain SRS30216^T was isolated utilizing the CTAB/NaCl procedure (Meade *et al.*, 1982). The 16S rRNA gene of strain SRS30216^T was amplified using the universal primers 27F (5′-AGA-

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The GenBank accession number for the 16S rRNA gene sequence of *Kineococcus radiotolerans* SRS30216^T is AF247813.

GTTTGATCMTGGCTCAG-3′; M = C/A) and 1392R (5′-ACGGGCGGTGTGTRC-3′; R = A/G) for the bacterial 16S rRNA gene (Wise *et al*., 1997). Both strands of the PCR product were sequenced (Molecular Genetics Instrumentation Facility, UGA, USA). The BLAST algorithm (Altschul *et al*., 1990) was used to identify the closest 16S rRNA gene matches present in GenBank. The closest relative of strain SRS30216^T is the type and only validly published species of the genus *Kineococcus*, *Kineococcus aurantiacus* RA 333^T (Yokota *et al*., 1993). The type strain of *K. aurantiacus*, a motile, coccus-shaped bacterium, was isolated from soil from the Indore region of India. Comparison of the 16S rRNA genes of strain SRS30216^T and *K. aurantiacus* RA 333^T utilizing the GAP program (GCG, Wisconsin Package) showed 93 % similarity over 1268 bp internal to the 16S rRNA gene. A higher level of similarity, $\geqslant$ 99 %, was observed between the 16S rRNA gene of strain SRS30216^T and the 16S rRNA genes of uncharacterized and not validly published Mojave Desert isolates AS3635, AS2960, AS3641, AS3079 and AS2987, whose sequences are also available in GenBank (accession numbers AF060694, AF060673, AF060695, AF060682 and AF060672, respectively).

A 1268 bp internal region of the amplified 16S rRNA gene sequence was used to perform phylogenetic analysis using PHYLIP version 3.5c (Felsenstein, 1993). Trees were constructed using the DNA distance and DNA parsimony methods (Hillis *et al*., 1993). Bootstrap analyses for 100 resamplings were performed with both algorithms to provide confidence estimates for tree topologies (Felsenstein, 1985). 16S rDNA sequences from closely associated organisms, based on sequence similarity determined by the BLAST algorithm, were included in the analysis. Phylogenetic trees constructed by DNA distance (Fig. 1) and DNA parsimony (data not shown) show that strain SRS30216^T and some of the uncharacterized bacteria from the Mojave Desert, such as AS3635, are more closely related to each other than to the type strain of *K. aurantiacus*. However, none of these strains were available to us for comparison.

DNA–DNA hybridization between strain SRS30216^T and the type strain of *K. aurantiacus* was performed at the Deutsche Sammlung von Mikrooganismen und Zellkulturen GmbH, Braunschweig, Germany. The hybridization conditions used were described by De Ley *et al*. (1970) with the modifications described by Huß *et al*. (1983) and Escara & Hutton (1980). DNA–DNA hybridization analysis between strain SRS30216^T and strain RA 333^T revealed only 31 % similarity.

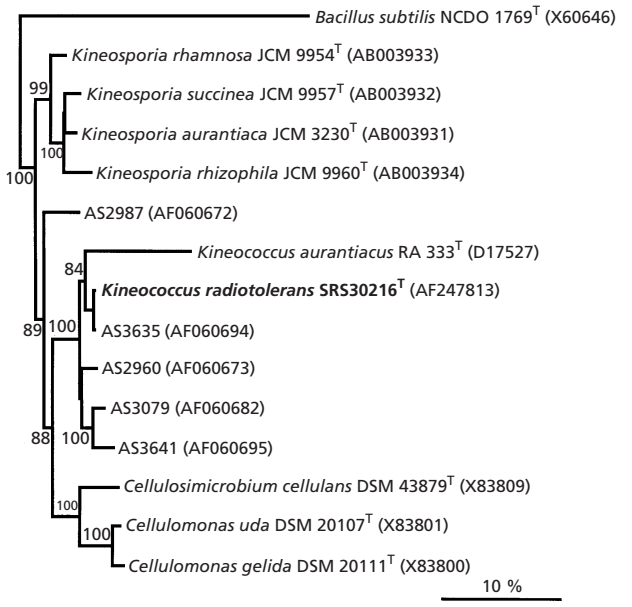

Fig. 1. Phylogenetic tree showing the position of strain *K. radiotolerans* SRS30216^T, *K. aurantiacus* RA 333^T and selected organisms within the *Actinobacteria*. The tree was constructed using the FITCH algorithm from a matrix of pairwise genetic distances as calculated by the Jukes–Cantor method. A total of 1250 aligned positions was used in the analysis. Bar, 0·10 substitutions per base position. The numbers at the nodes of the tree indicate the number of times the group consisting of the species listed to the right of that fork occurred among 100 bootstrapped resamplings (values below 60 are not shown). The accession number for each organism is given in parentheses.

Morphological and cultural characteristics

Strain SRS30216^T colonies were orange and round with rough edges. Individual cells were coccus shaped, approximately 1·0–1·5 μm in diameter. Within a broth culture, approximately 1 % of the cells were observed to be motile. Motility was stimulated in broth culture by incubation of cells in a solution of 10 % sandy loam soil extract (Lennette *et al*., 1985) for 1 h; in this case, the number of motile cells increased to nearly 100 %. Motility was also observed as spreading colonies on yeast extract/malt extract plates [0·4 % (w/v) yeast extract, 1 % (w/v) malt extract, 0·4 % (w/v) glucose, 0·3 % (w/v) Bacto agar] incubated at 32 °C for 3 days. Scanning electron microscopy was performed to visualize cell morphology and flagella production. For cell morphology, cells were collected from broth cultures by centrifugation or scraped from a plate, washed once in 67 mM phosphate buffer (4·73 g Na_2HPO_4 l^{-1}, 4·5 g KH_2PO_4 l^{-1}, pH 7·0) and resuspended in 100 μl of the same buffer. An equal volume of 4 % (v/v) glutaraldehyde in 0·1 M cacodylate buffer was added to the cell suspension for 1 h at room temperature. The cells were then washed three times with phosphate buffer and collected on nitrocellulose filters with a 1 μm pore size (Millipore) before being serially dehydrated with ethanol using 20, 40, 60 and 80 % (v/v) steps ending in three changes at 100 %. Critical-point drying (Samdri) of the samples was performed before coating with chromium using a vacuum evaporator (Edwards) and observation with a

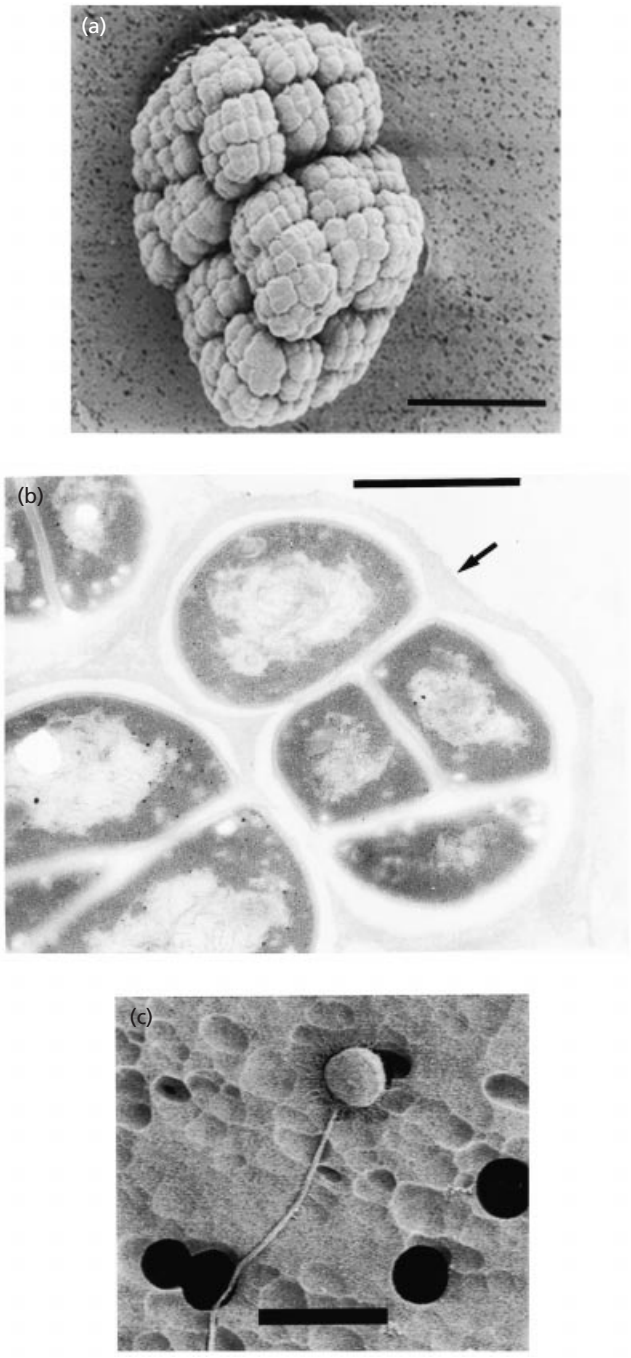

Fig. 2. (a) SEM of *K. radiotolerans* SRS30216^T cultured on PTYG agar (pH 7·0) showing the clusters formed by growing cells. Bar, 10 μm. (b) TEM of a thin section of cells of strain SRS30216^T. Arrow shows the presence of extracellular matrix. Bar, 1 μm. (c) SEM of a motile cell of strain SRS30216^T exhibiting a single flagellum. Bar, 2 μm.

LEO 982 field emission scanning electron microscope. When grown on plates or in broth, the cells grew in symmetrical clumps (Fig. 2a). For transmission electron microscopy, glutaraldehyde-fixed cells were embedded with Epon resin (Electron Microscopy Science) and polymerized at 60 °C for 18 h. Seventy to eighty micrometre sections were cut with an RMC 6000 ultramicrotome (Ventana Medical Instruments) and viewed on a JEOL 100CX operating at 80 kV. Thin sections revealed clumps of cells surrounded by an extracellular matrix or slime layer (Fig. 2b). This cell-surface component was more apparent when cells were grown in broth (data not shown). Cells containing flagella were visualized by scanning electron microscopy using cells previously incubated in soil extract to induce motility before fixing with glutaraldehyde. Motile cells were more spherical than cells that were part of clusters (Fig. 2c).

Both strain SRS30216^T and *K. aurantiacus* RA 333^T produced an orange pigment that is soluble in methanol, thus allowing comparison of the pigments by absorption spectrum. Cultures of both strains were washed once with H_2O, resuspended in 100 % methanol and vortexed vigorously for 5 min. After centrifugation at 12000 ***g*** for 5 min, the methanol extract was removed and the visible light absorption spectrum was obtained from 340 to 600 nm using a Beckman DU640B spectrometer. Both pigment extracts contained absorption peaks at approximately 444, 471 and 501 nm, suggesting a carotenoid (Kleinig *et al.*, 1970).

Physiological characterization

Like *K. aurantiacus* RA 333^T, strain SRS30216^T stained Gram-positive. Catalase activity was observed when a solution of 3 % (v/v) hydrogen peroxide was dropped onto cells placed on a glass slide. No oxidase activity was seen in an assay involving reduction of 1 % tetramethyl-*p*-phenylenediamine previously placed on filter paper disks. Unlike *K. aurantiacus* RA 333^T, however, urease activity was not observed on a urease slant. The temperature range for growth was determined in PTYG broth in a temperature gradient incubator set with low and high temperatures of 0 and 55 °C. A growing culture of strain SRS30216^T was diluted into fresh medium to an OD_{600} of less than 0·1. A tenfold increase in optical density was considered positive for growth. Observation of cultures over 96 h revealed growth in PTYG broth over a temperature range of 11 to 41 °C. The doubling time at 32 °C was 2·5 h. These characteristics are comparable to those of *K. aurantiacus* RA 333^T.

For pH range and salt tolerance experiments, exponential phase cells were diluted 1:500 into the appropriate medium and incubated at 32 °C. A doubling of cell mass over the course of 3 days was considered positive. To determine the range of pH that would allow growth of strain SRS30216^T, cells were incubated in PTYG broth at a specific pH at 32 °C with aeration. The pH of the medium was measured both before and after growth to ensure that the pH had been maintained. As with *K. aurantiacus* RA 333^T, growth of strain SRS30216^T was observed between pH 5 and 9, but not at pH 4·5 or 9·5 in PTYG. Growth in the

presence of salt was determined by the addition of NaCl to PTYG broth to produce a series of concentrations from 0 to 7% (w/v) in 0·5% increments. Growth was observed at salt concentrations up to and including 5%. To determine the ability of the organism to use different carbon sources, cells were scraped from PTYG plates and resuspended in 0·5% (w/v) yeast extract. Different carbon sources were added at 0·5% (w/v) and the cultures were incubated for 3 days. Utilization of the carbon source was deemed positive if the cell density was at least double the density of the control culture, which contained no added carbon source. Strain SRS30216^T utilized glucose, galactose, L-arabinose, sucrose, mannose, xylose, glycerol, mannitol, inositol and sorbitol as carbon sources. Raffinose, rhamnose, lactose, ribose and maltose were unable to stimulate growth. The Simmons citrate test (Lennette *et al.*, 1985) was negative. Strain SRS30216^T was unable to utilize ribose and citrate, thus differentiating it from *K. aurantiacus* RA 333^T.

Biochemical analysis

Fatty acid analysis was performed by Microbial ID based on GC column retention time using extracts from cells grown on TSBA [3% (w/v) tryptic soy broth with 1·5% (w/v) Bacto agar] at 30 °C. With both strains, the majority of fatty acids (> 90%) consisted of anteiso 15:0. This is similar to the value of 88·7% reported for *K. aurantiacus* RA 333^T by Yokota *et al.* (1993). The remaining fatty acids had chain lengths between 14 and 18 and were found in similar percentages in strain SRS30216^T and *K. aurantiacus* RA 333^T (data not shown). Surprisingly, the results suggested that strain SRS30216^T produced the polyunsaturated fatty acid 20:4ω6,9,12,15*c* (arachidonic acid). The identification of polyunsaturated fatty acids produced by bacteria has been limited to organisms isolated from marine psychrophilic environments (Russell & Nichols, 1999). This unusual finding prompted a closer examination of the lipid and fatty acid composition of strain SRS30216^T using MS at the Center for Biomarker Analysis (Knoxville, TN, USA). Cells were grown in PTYG broth at 15, 23 and 37 °C and the lipids were extracted after purification from lyophilized cells (Bligh & Dyer, 1959; White *et al.*, 1979). The lipids were fractionated into polar, neutral and glycolipids by sequential elution from a silicic acid column (Guckert *et al.*, 1985). Fatty acid methyl esters were identified by GC-MS of samples using a Hewlett Packard 6890 series GC interfaced to a Hewlett Packard 5973 mass selective detector. Again, the vast majority of the fatty acids were anteiso 15:0, regardless of the chemical nature of the lipid (polar, neutral or glycolipid) or growth temperature. Arachidonic acid was not detected and the peak corresponding to it in the MIDI analysis was probably an alkene. Interestingly, when strain SRS30216^T was grown at 15 °C, no neutral lipids were produced; instead, this fraction was composed entirely of alkenes. Alkenes were produced at all three temperatures and were composed of a variety of species with chain lengths of 19–24 carbons. One alkene containing 21 carbons and one alkene containing 22 carbons together constituted approximately 70% of the total alkene production. The exact nature of these compounds has not been investigated.

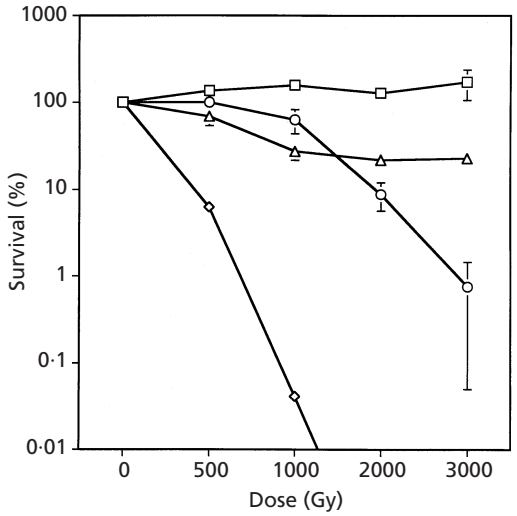

Fig. 3. Resistance to γ-radiation from a ^{60}Co source. □, *D. radiodurans* ATCC 13939^T; ◇, *E. coli* CF1648 (*recA*$^+$); ○, *K. aurantiacus* RA 333^T; and △, *K. radiotolerans* SRS30216^T. Error bars represent the standard deviation; some standard deviations are too small to be seen on the graph. The graph is representative of results obtained on three different occasions.

Radiation resistance

Since strain SRS30216^T was isolated from a radioactive work area, the radiation resistance of this strain was compared to that of *K. aurantiacus* RA 333^T and the radiation-resistant organism *Deinococcus radiodurans* ATCC 13939^T. Exponentially growing cultures of *D. radiodurans* ATCC 13939^T, *Escherichia coli* CF1648 (*recA*$^+$) [obtained from M. Cashel (NIH, Bethesda, MA, USA) and used as a radiation-sensitive control], strain SRS30216^T and *K. aurantiacus* RA 333^T were washed and resuspended in an equal volume of 67 mM phosphate buffer and divided into 100 µl aliquots. The cell suspensions were exposed to a ^{60}Co source for predetermined times. At each time-point, three individual aliquots of each strain were removed from the radiation source. Cell suspensions were serial diluted in 67 mM phosphate buffer and plated on PTYG medium. After 3 days growth, c.f.u. were counted and the percentage survival was calculated based upon the number of c.f.u. present before irradiation. *K. aurantiacus* RA 333^T showed an intermediate level of radiation resistance compared with *D. radiodurans* ATCC 13939^T and *E. coli* CF1648, but was much less resistant than SRS30216^T (Fig. 3). In fact, no logarithmic killing of strain SRS30216^T was observed at doses up to 3·5 kGy and less than a 1 log

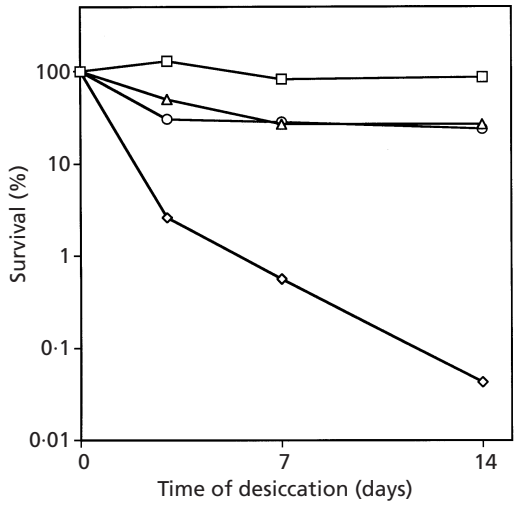

Fig. 4. Resistance to desiccation. □, *D. radiodurans* ATCC 13939^T; ◇, *E. coli* CF1648 (*recA*$^+$); ○, *K. aurantiacus* RA 333^T; △, *K. radiotolerans* SRS30216^T. The graph is representative of results obtained on three different occasions.

difference was observed between strain SRS30216^T and *D. radiodurans* ATCC 13939^T at 3·5 kGy.

Desiccation resistance

Because a correlation has been made between desiccation resistance and radiation resistance (Mattimore & Battista, 1996), strain SRS30216^T was tested for desiccation resistance. Exponentially growing cultures were washed once and resuspended in an equal volume of 67 mM phosphate buffer. Aliquots (1 ml) of cultures of *D. radiodurans* ATCC 13939^T, *E. coli*, strain SRS30216^T and *K. aurantiacus* RA 333^T were placed onto glass cover-slips (1 inch × 1 inch). The cover-slips were then placed in sterile Petri dishes inside a vacuum desiccator containing calcium sulfate. An electronic hygrometer (Fisher Scientific) measured the humidity as 7±2% at 25 °C. The percentage survival for each strain was determined at 3, 7 and 14 days after desiccation. At each time-point, one cover-slip containing each strain was removed. Phosphate buffer (1 ml) was added to the cover-slips to rehydrate the cells. Tenfold serial dilution and plating was then used to determine the percentage survival. Over a 2 week period, *D. radiodurans* ATCC 13939^T showed the most resistance and *E. coli* CF1648 showed the least resistance. SRS30216^T and RA 333^T were similar and only slightly less resistant than *D. radiodurans* ATCC 13939^T (Fig. 4).

In conclusion, strain SRS30216^T shows 93% 16S rDNA sequence identity to *K. aurantiacus* RA 333^T. Furthermore, DNA–DNA hybridization experiments revealed only 31% DNA similarity between strain SRS30216^T and *K. aurantiacus* RA 333^T. Strain SRS30216^T was much more resistant to γ-radiation than *K. aurantiacus* RA 333^T. Although strain SRS30216^T is very similar to *K. aurantiacus* RA 333^T, it differs enough in 16S rDNA sequence and DNA similarity by DNA–DNA hybridization to be considered a separate species. The original description of *K. aurantiacus* RA 333^T suggests that this genus should be included in the family *Pseudonocardiaceae* (Embley *et al*., 1988). One of the main properties of this family is the production of a majority of iso- and anteiso-branched chain fatty acids. The fatty acid composition of strain SRS30216^T (mainly anteiso 15:0) is consistent with the inclusion of this organism in the family *Pseudonocardiaceae*. However, the proposed 16S rDNA signature sequence for *Pseudonocardiaceae* (Stackebrandt *et al*., 1997) is not conserved in SRS30216^T, with differences at 12 of 20 positions (data not shown). *K. aurantiacus* RA 333^T also poorly matched the signature sequence. Perhaps this means the signature sequence for the family needs to be reconsidered in light of this novel genus and species.

It is proposed that the novel isolate be placed in the genus *Kineococcus* as a novel species, *Kineococcus radiotolerans* sp. nov.

Description of *Kineococcus radiotolerans* sp. nov.

Kineococcus radiotolerans (ra.di.o.to′le.rans. L. n. *radiatio* radiation; L. part. adj. *tolerans* tolerating; N.L. adj. *radiotolerans* radiation-tolerating).

Cells are cocci, 1·0–1·5 μm in diameter. Cells occur in pairs, tetrads and in larger clusters. Colonies are circular, rough and orange-pigmented. Gram-reaction positive. Cells are motile and produce polar flagella. Catalase-positive. Urease and oxidase tests are negative. A variety of carbon sources are used including glucose, galactose, L-arabinose, sucrose, mannose, xylose, glycerol, mannitol, inositol and sorbitol, but not raffinose, rhamnose, lactose, citrate, ribose or maltose. The major fatty acid produced is anteiso 15:0 (approx. 90%). An orange pigment, soluble in methanol, with an absorption spectrum containing peaks at 444, 471 and 501 nm, is produced. The type and only strain is SRS30216^T (= ATCC BAA-149^T = DSM 14245^T), which was isolated from the Savannah River Site in Aiken, South Carolina, USA.

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References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). A basic local alignment search tool. *J Mol Biol* **215**, 403–410.

Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction. *Can J Biochem Physiol* **37**, 911–917.

De Ley, P., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Embley, M. T., Smida, J. & Stackebrandt, E. (1988). The phylogeny of mycolate-less chemotype IV actinomycetes and description of *Pseudonocardiaceae* fam. nov. *Syst Appl Microbiol* **11**, 44–52.

Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethyl sulfoxide solutions: acceleration of the renaturation rate. *Biopolymers* **19**, 1315–1327.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Felsenstein, J. (1993). PHYLIP (phylogenetic inference package), version 3.5c. Department of Genetics, University of Washington, Seattle, WA, USA.

Guckert, J. B., Antworth, C. P., Nichols, P. D. & White, D. C. (1985). Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. *FEMS Microbiol Ecol* **31**, 147–158.

Hillis, D. M., Allard, M. W. & Miyamoto, M. M. (1993). Analysis of DNA sequence data: phylogenetic inference. In *Molecular Evolution: Producing the Biochemical Data*, pp. 456–487. Edited by E. A. Zimmer, T. J. White, R. L. Cann & A. C. Wilson. San Diego, CA: Academic Press.

Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies of the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Kleinig, H., Reichenbach, H. & Achenbach, H. (1970). Carotenoid pigments of *Stigmatella aurantiaca* (Myxobacterales). II. Acylated carotenoid glucosides. *Arch Mikrobiol* **74**, 223–234.

Lennette, E. H., Balows, A., Hausler, W. J., Jr & Shadomy, H. J. (1985). *Manual of Clinical Microbiology*, 4th edn. Washington, DC: American Society for Microbiology.

Mattimore, V. & Battista, J. R. (1996). Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. *J Bacteriol* **178**, 633–637.

Meade, H. M., Long, S. R., Ruvkun, C. B., Brown, S. E. & Ausubel, F. M. (1982). Physical and genetic characterization of symbiotic and auxotrophic mutants of *Rhizobium meliloti* induced by transposon Tn5 mutagenesis. *J Bacteriol* **149**, 114–122.

Russell, N. J. & Nichols, D. S. (1999). Polyunsaturated fatty acids in marine bacteria – a dogma rewritten. *Microbiology* **145**, 767–779.

Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.

White, D. C., Bobbie, R. J., Heron, J. S., King, J. D. & Morrison, S. J. (1979). Biochemical measurements of microbial mass and activity from environmental samples. In *Native Aquatic Bacteria: Enumeration, Activity, and Ecology*, pp. 69–81. Edited by J. W. Costerton & R. R. Colwell. Philadelphia, PA: American Society for Testing and Materials.

Wise, M. G., McArthur, J. V. & Shimkets, L. J. (1997). Bacterial diversity of a Carolina bay as determined by 16S rRNA gene analysis: confirmation of novel taxa. *Appl Environ Microbiol* **63**, 1505–1514.

Yokota, A., Tamura, T., Nishii, T. & Hasegawa, T. (1993). *Kineococcus aurantiacus* gen. nov., sp. nov., a new aerobic, gram-positive, motile coccus with *meso*-diaminopimelic acid and arabinogalactan in the cell wall. *Int J Syst Bacteriol* **43**, 52–57.