**INTRODUCTION**

Tidal flats are known to be a good environment from which to isolate novel and useful micro-organisms (Yi et al., 2003; Yoon et al., 2003c). Some new genera and species have recently been isolated from tidal flats in Korea (Yi et al., 2003; Yoon et al., 2003c, f). In the course of screening novel micro-organisms from a tidal flat of the Yellow Sea in Korea, many moderately halophilic or halotolerant bacteria have been isolated and characterized taxonomically. Among these isolates, two bacterial strains, SW-125T and SW-154T, were investigated in detail here because they were found to form deep branches within the class *Proteobacteria* in phylogenetic analyses based on 16S rRNA gene sequences. Accordingly, the aim of this work was to determine the exact taxonomic positions of strains SW-125T and SW-154T using a polyphasic characterization that included phenotypic properties, detailed phylogenetic analyses based on 16S rRNA gene sequences and genomic relatedness. On the basis of the data presented, it is proposed that strains SW-125T and SW-154T should be placed in a new genus, *Kangiella* gen. nov., in two distinct novel species, *Kangiella koreensis* sp. nov. and *Kangiella aquimarina* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea.

**METHODS**

**Bacterial strains and cultural conditions.** Tidal flat sediment was collected from Daepo Beach, Yellow Sea, Korea, and used as a source for isolation of bacterial strains. Strains SW-125T and SW-154T were isolated from tidal flat sediment of the Yellow Sea in Korea, and subjected to a polyphasic taxonomic study. Strains SW-125T and SW-154T grew optimally at 30–37 °C and in the presence of 2–3 % (w/v) NaCl. They contained ubiquinone-8 (Q-8) as the predominant respiratory lipoquinone and iso-C₁₅ : 0 as the major fatty acid. The DNA G+C contents of strains SW-125T and SW-154T were 44 mol%. Phylogenetic trees based on 16S rRNA gene sequences revealed that the two strains form deep evolutionary lineages of descent within the *γ-Proteobacteria*. Strains SW-125T and SW-154T exhibited 16S rRNA gene sequence similarity levels of less than 90 % to members of the *γ-Proteobacteria* used in this analysis. Strains SW-125T and SW-154T showed a 16S rRNA gene sequence similarity level of 98-5 % and a mean DNA–DNA relatedness level of 9.4 %. Therefore, on the basis of phenotypic, phylogenetic and genomic data, a new genus, *Kangiella* gen. nov., is proposed to accommodate the novel strains, comprising two novel species, *Kangiella koreensis* sp. nov. (type strain, SW-125T =DSM 16069T) and *Kangiella aquimarina* sp. nov. (type strain, SW-154T =KCTC 12182T =DSM 16069T).

*Kangiella koreensis* gen. nov., sp. nov. and *Kangiella aquimarina* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea

Jung-Hoon Yoon, Tae-Kwang Oh and Yong-Ha Park

Korea Research Institute of Bioscience and Biotechnology (KRIIBB), PO Box 115, Yusong, Taejon, Korea

Two Gram-negative, non-motile, non-spore-forming, rod-shaped organisms, strains SW-125T and SW-154T, were isolated from tidal flat sediment of the Yellow Sea in Korea, and subjected to a polyphasic taxonomic study. Strains SW-125T and SW-154T grew optimally at 30–37 °C and in the presence of 2–3 % (w/v) NaCl. They contained ubiquinone-8 (Q-8) as the predominant respiratory lipoquinone and iso-C₁₅ : 0 as the major fatty acid. The DNA G+C contents of strains SW-125T and SW-154T were 44 mol%. Phylogenetic trees based on 16S rRNA gene sequences revealed that the two strains form deep evolutionary lineages of descent within the *γ-Proteobacteria*. Strains SW-125T and SW-154T exhibited 16S rRNA gene sequence similarity levels of less than 90 % to members of the *γ-Proteobacteria* used in this analysis. Strains SW-125T and SW-154T showed a 16S rRNA gene sequence similarity level of 98-5 % and a mean DNA–DNA relatedness level of 9.4 %. Therefore, on the basis of phenotypic, phylogenetic and genomic data, a new genus, *Kangiella* gen. nov., is proposed to accommodate the novel strains, comprising two novel species, *Kangiella koreensis* sp. nov. (type strain, SW-125T =DSM 16069T) and *Kangiella aquimarina* sp. nov. (type strain, SW-154T =KCTC 12182T =DSM 16071T).
nitrate that had been prepared anaerobically. Catalase activity was determined by bubble production in a 3% (v/v) hydrogen peroxide solution. Oxidase activity was determined by oxidation of 1% p-amidinomethylaniiline oxalate. Hydrolysis of casein, starch, Tween 20, Tween 40, Tween 60 and Tween 80 was determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and gelatin and nitrate reduction were tested as described previously (Lanyi, 1987) with a modification that artificial sea water was used. The artificial sea water contained (per litre distilled water) 23·6 g NaCl, 0·64 g KCl, 4·53 g MgCl2.6H2O, 5·94 g MgSO4.7H2O and 1·3 g CaCl2.2H2O (Levring, 1946). Hydrolysis of hydroxytine, tyrosine and xanthine was tested on MA plates using the substrate concentrations given by Cowan & Steel (1965). H2S production was tested as described by Bruns et al. (2001). Acid production from carbohydrates was determined as described by Leifson (1963). Enzyme activity was determined using the API ZYM system (bioMérieux). Requirements of peptone, yeast extract, Hutner’s mineral solution (Cohen-Bazire et al., 1957) and vitamin solution (Staley, 1968) for growth were determined in the liquid marine salts basal media (Baumann & Baumann, 1981) containing 0·1% each of glucose, sucrose and acetate at the following concentrations: peptone (0·05%), yeast extract (0·01%), mineral solution (2%, v/v) and vitamin solution (1%, v/v). Susceptibility to antibiotics was determined on agar plates using antibiotic discs with the following concentrations: polymyxin B (50 U), streptomycin (50 μg), penicillin (20 U), chloramphenicol (50 μg), ampicillin (10 μg), cephalothin (30 μg), gentamicin (30 μg), novobiocin (5 μg), erythromycin (15 μg) and tetracycline (30 μg).

**Chemotaxonomic characterization.** Chromosomal DNA was isolated and purified according to the method described by Yoon et al. (1996), except that ribonuclease T1 was used together with ribonuclease A to minimize the contamination of RNA. Respiratory lipoquinones were analysed as described by Komaga & Suzuki (1987) using reversed-phase HPLC. For quantitative analysis of cellular fatty acid composition, a loop of cell mass was harvested and FAMEs were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984). DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

**DNA–DNA hybridization.** This was performed fluorometrically by the method of Ezaki et al. (1989), using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. Of the values obtained, the highest and lowest values for each sample were excluded; DNA–DNA relatedness values are the mean of the remaining three values.

**16S rRNA gene sequencing and phylogenetic analyses.** The 16S rRNA gene was amplified by PCR using two universal primers as described by Yoon et al. (1998). The PCR product was purified with a QIAquick PCR purification kit (Qiagen). Sequencing of the purified 16S rRNA gene PCR product was performed using an ABI PRISM BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems) according to the manufacturer’s recommendations. The purified sequencing reaction mixtures were electrophoresed automatically using an Applied Biosystems model 377 automatic DNA sequencer. Alignment of sequences was carried out using CLUSTAL W software (Thompson et al., 1994), and the resulting alignment was then modified to remove regions containing ambiguous bases and gaps. Phylogenetic trees were inferred by using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods contained in the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices for the neighbour-joining method were calculated using the algorithm of Jukes & Cantor (1969) with the program DNADIST. The stability of relationships was assessed by a bootstrap analysis, based on 1000 resamplings of the neighbour-joining dataset, using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package.

## RESULTS AND DISCUSSION

### Phenotypic characteristics

Strains SW-125T and SW-154T grew optimally at 30–37°C. Strain SW-125T grew at 4°C; strain SW-154T did not grow at 4°C but did grow at 10°C. Maximum growth temperatures of strains SW-125T and SW-154T were 43 and 48°C, respectively. Both strains required peptone for growth. Acid phosphatase is present in strain SW-154T, but not in strain SW-125T. Both strains contained ubiquinone-8 (Q-8) as the predominant respiratory lipoquinone at peak ratios of approximately 84–88%. Strains SW-125T and SW-154T had cellular fatty acid profiles containing large amounts of branched fatty acids, particularly iso-branched fatty acids (Table 1). The major fatty acid detected in the two strains was iso-C15:0 (Table 1). The DNA G+C contents of strains SW-125T and SW-154T were both 44 mol%. Other phenotypic properties of the two strains are given in the species descriptions below or are shown in Table 2.

### Phylogeny and DNA–DNA relatedness

The almost-complete 16S rRNA gene sequences of strains SW-125T and SW-154T determined in this study comprised 1494 and 1496 nucleotides, respectively, representing approximately 96% of the *Escherichia coli* 16S rRNA sequence. The level of 16S rRNA gene sequence similarity

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>SW-125T</th>
<th>SW-154T</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>iso-C11:0</td>
<td>5.6</td>
<td>6.5</td>
</tr>
<tr>
<td>iso-C13:0</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>57.6</td>
<td>50.7</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>7.2</td>
<td>11.0</td>
</tr>
<tr>
<td>iso-C17:1 ω9c</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>iso-C11:0 3 OH</td>
<td>10.5</td>
<td>11.7</td>
</tr>
<tr>
<td>iso-C15:0 3 OH</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>iso-C17:0 3 OH</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Summed feature 1†</td>
<td>3.2</td>
<td>4.0</td>
</tr>
</tbody>
</table>

*The double bond positions indicated by upper-case letters are unknown.
†Summed feature 1 represents iso-C15:1 and/or C13:0 3 OH, which could not be separated by GLC with the MIDI system.
between strains SW-125\textsuperscript{T} and SW-154\textsuperscript{T} was 98.5\%.

Phylogenetic trees based on 16S rRNA gene sequences revealed that the two strains form independent phylogenetic lineages within the evolutionary radiation encircled by members of the \(\gamma\)-Proteobacteria (Fig. 1). In the phylogenetic tree based on the neighbour-joining algorithm, strains SW-125\textsuperscript{T} and SW-154\textsuperscript{T} formed a cluster supported by a bootstrap resampling value of 100\%, and joined the phylogenetic clade comprising authentic pseudomonads and several genera, Marinobacterium, Marinomonas, Oceanospirillum, Pseudospirillum, Shewanella, Alteromonas, Oceanobacter, Halomonas, Marinospirillum, Microbulbifer, Marinobacter and Alcanivorax, by a bootstrap resampling value of 61.1\% (Fig. 1). Strains SW-125\textsuperscript{T} and SW-154\textsuperscript{T} exhibited 16S rRNA gene sequence similarity levels of less than 89.3\% (Microbulbifer hydrolyticus ATCC 700072\textsuperscript{T}) and 89.0\% (Microbulbifer hydrolyticus ATCC 700072\textsuperscript{T} and Oceanobacter kriegii IFO 15467\textsuperscript{T}), respectively, to species used in the phylogenetic analysis (Fig. 1). Strains SW-125\textsuperscript{T} and SW-154\textsuperscript{T} exhibited a mean level of DNA–DNA relatedness of 9.4\%, when their DNA was used individually as a labelled DNA probe, indicating that the two strains represent members of different genomic species (Wayne et al., 1987).

### Table 2. Differential phenotypic properties of the genus Kangiella and some other related genera

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods or cocci</td>
<td>Rods</td>
<td>Rods</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>V</td>
<td>+</td>
<td>– or ND</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>–†</td>
<td>– or ND</td>
<td>V</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Anaerobic growth</td>
<td>+</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aesculin</td>
<td>–</td>
<td>–†</td>
<td>– or ND</td>
<td>V</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>–†</td>
<td>V</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>–†</td>
<td>V</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tween 80</td>
<td>+</td>
<td>+†</td>
<td>+ or ND</td>
<td>+</td>
<td>+‡</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 45 °C</td>
<td>V</td>
<td>–</td>
<td>V</td>
<td>V</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth at 4 °C</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>–</td>
<td>V</td>
<td>–</td>
</tr>
<tr>
<td>Growth at 10% NaCl</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+‡</td>
<td>ND</td>
</tr>
<tr>
<td>Predominant ubiquinone</td>
<td>Q-8</td>
<td>ND</td>
<td>Q-9</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8</td>
</tr>
<tr>
<td>Major fatty acid(s)</td>
<td>iso-C\textsubscript{15.0}</td>
<td>C\textsubscript{16-0}</td>
<td>C\textsubscript{18-1}</td>
<td>C\textsubscript{18-1} o9c</td>
<td>iso-C\textsubscript{15-0}</td>
<td>iso-C\textsubscript{17-1}</td>
</tr>
<tr>
<td></td>
<td>C\textsubscript{18-1} o9c</td>
<td>iso-C\textsubscript{16-0}</td>
<td>iso-C\textsubscript{17-1}</td>
<td>C\textsubscript{16-0}</td>
<td>C\textsubscript{16-1}</td>
<td>C\textsubscript{18-1}</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>44</td>
<td>53–66</td>
<td>53–64</td>
<td>58–60</td>
<td>55–60</td>
<td>54–56</td>
</tr>
</tbody>
</table>

*Positive under anaerobic conditions.†Data for Alcanivorax borkumensis.
‡Data for Marinobacterium georgiense.

### Conclusion

The phylogenetic analyses based on 16S rRNA gene sequences indicated that strains SW-125\textsuperscript{T} and SW-154\textsuperscript{T} did not fall within the radiation encompassed by a recognized genus but form distinct lineages within the \(\gamma\)-Proteobacteria. The 16S rRNA gene sequence similarity values to members of the \(\gamma\)-Proteobacteria were very low (less than 90\%). Strains SW-125\textsuperscript{T} and SW-154\textsuperscript{T} are differentiated from some phylogenetically related genera by several phenotypic characteristics, particularly respiratory lipoquinone and fatty acid profiles (Table 2). The genera Pseudomonas, Halomonas and Marinobacter have been observed to have ubiquinone-9 as the predominant respiratory lipoquinone (Oyaizu & Komagata, 1983; Dobson & Franzmann, 1996; Yumoto et al., 2001; Yoon et al., 2003b). Shewanella species simultaneously contain both menaquinones and ubiquinones (Venkateswaran et al., 1999; Bozal et al., 2002). The fatty acid patterns, particularly the major fatty acid(s), distinguish strains SW-125\textsuperscript{T} and SW-154\textsuperscript{T} from some genera, including Alcanivorax, Alteromonas, Microbulbifer, Marinobacterium, Marinomonas, Methyllococcus, Methylocrubium, Oceanospirillum and Oceanobacter (Bowman et al., 1993; González et al., 1997; Fernández-Martínez et al., 2003; Satomi et al.,
Therefore, it is appropriate that strains SW-125T and SW-154T are allocated in a new genus within the \textit{c-Proteobacteria}. Although strains SW-125T and SW-154T are similar in most phenotypic properties, they are considered to belong to different species on the basis of DNA–DNA relatedness and differences in their phenotypic properties, such as temperature for growth. On the basis of the phenotypic and phylogenetic data and the genomic distinctiveness, strains SW-125T and SW-154T should be placed in a new genus, \textit{Kangiella} gen. nov., in two novel species, \textit{Kangiella koreensis} sp. nov. and \textit{Kangiella aquimarina} sp. nov., respectively.

\textbf{Description of \textit{Kangiella} gen. nov.}

\textit{Kangiella} (Kan.gi.el\textit{la}, N.L. dim. fem. n. \textit{Kangiella} named to honour Professor Kook Hee Kang, a Korean microbiologist, for his contribution to microbial research).

Gram-negative, non-motile, non-spore-forming rods.
Colonies are smooth, raised, circular to slightly irregular and light brown in colour on MA. Catalase- and oxidase-positive. Urease-negative. Nitrate reduction is negative under aerobic conditions. Nitrate is reduced to nitrogen gas under anaerobic conditions. NaCl and peptone are required for growth. The predominant respiratory lipoquinone is ubiquinone-8 (Q-8). The major fatty acid is iso-C_{15:0}. The DNA G+C content is 44 mol% (as determined by HPLC).

The type species is *Kangiella koreensis*.

**Description of *Kangiella koreensis* sp. nov.**

*Kangiella koreensis* (ko.re.en’sis. N.L. fem. adj. koreensis pertaining to Korea, from where the organism was isolated).

Cells are rods, 0·2–0·5 × 1·5–4·5 μm. Non-motile. Non-spor-forming. Gram-negative. Colonies are smooth, raised, circular to irregular, light yellowish-brown in colour and 2·0–3·0 mm in diameter after 7 days incubation at 30°C on MA. Optimal growth temperature is 30–37°C. Growth occurs at 4°C. Maximum growth temperature is 43°C. Optimal growth pH is 7·0–8·0. Growth occurs at pH 5·5, but not at pH 5·0. Optimal growth occurs in the presence of 2–3% NaCl. Growth occurs in the presence of 12% NaCl, but not without NaCl or in the presence of more than 13% NaCl. Growth under anaerobic conditions occurs on MA supplemented with nitrate. Casein, tyrosine, Tween 20, Tween 40 and Tween 60 are hydrolysed. Hypoxanthine and xanthine are not hydrolysed. H$_2$S is not produced. Nitrate is not reduced under aerobic conditions. Nitrate is reduced to nitrogen gas under anaerobic conditions. Acid is not produced from the following sugars: adonitol, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannitol, D-mannose, D-melezitose, melibiose, myo-inositol, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, sucrose, D-trehalose or D-xyllose. When assayed with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase are present, but lipase (C14), β-galactosidase, β-glucosidase, β-glucuronidase, β-glucosidase, β-glucosidase, β-glucosidase, β-glucosidase, N-acetyl-β-mannosaminidase, β-mannosidase and α-fucosidase are absent. Susceptible to polymyxin (50 μg), streptomycin (50 μg), penicillin (20 μg), chloramphenicol (50 μg), ampicillin (10 μg), cephalothin (30 μg) and erythromycin (15 μg). Resistant to novobiocin (5 μg) and tetracycline (30 μg). The predominant respiratory lipoquinone is ubiquinone-8 (Q-8). The major fatty acid is iso-C$_{15:0}$. The DNA G+C content is 44 mol% (as determined by HPLC).

The type strain (SW-154$^T$ = KCTC 12183$^T$ = DSM 16071$^T$) was isolated from tidal flat sediment of the Yellow Sea, Korea.

**Description of *Kangiella aquimarina* sp. nov.**

*Kangiella aquimarina* (a.qui.ma.ri.na. L. n. aqua water; L. adj. marinus of the sea; N.L. fem. adj. aquimarina pertaining to sea water).

Cells are rods, 0·2–0·5 × 1·5–4·5 μm. Non-motile. Non-spor-forming. Gram-negative. Colonies are smooth, raised, circular to irregular, light yellowish-brown in colour and 2·0–3·0 mm in diameter after 7 days incubation at 30°C on MA. Optimal growth temperature is 30–37°C. Growth occurs at 10°C, but not at 4°C. Maximum growth temperature is 48°C. Optimal growth pH is 7·0–8·0. Growth occurs at pH 5·5, but not at pH 5·0. Optimal growth occurs in the presence of 2–3% NaCl. Growth occurs in the presence of 12% NaCl, but not without NaCl or in the presence of more than 13% NaCl. Growth under anaerobic conditions occurs on MA supplemented with nitrate. Casein, tyrosine, Tween 20, Tween 40 and Tween 60 are hydrolysed. Hypoxanthine and xanthine are not hydrolysed. H$_2$S is not produced. Nitrate is not reduced under aerobic conditions. Nitrate is reduced to nitrogen gas under anaerobic conditions. Acid is not produced from the following sugars: adonitol, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannitol, D-mannose, D-melezitose, melibiose, myo-inositol, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, sucrose, D-trehalose or D-xyllose. When assayed with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, phosphatase and naphthol-AS-BI-phosphohydrolase are present, but lipase (C14), β-galactosidase, β-glucosidase, β-glucosidase, β-glucosidase, β-glucosidase, N-acetyl-β-mannosaminidase, β-mannosidase and α-fucosidase are absent. Susceptible to polymyxin (50 μg), streptomycin (50 μg), penicillin (20 μg), chloramphenicol (50 μg), ampicillin (10 μg), cephalothin (30 μg) and erythromycin (15 μg). Resistant to novobiocin (5 μg) and tetracycline (30 μg). The predominant respiratory lipoquinone is ubiquinone-8 (Q-8). The major fatty acid is iso-C$_{15:0}$. The DNA G+C content is 44 mol% (as determined by HPLC).

The type strain (SW-154$^T$ = KCTC 12183$^T$ = DSM 16071$^T$) was isolated from tidal flat sediment of the Yellow Sea, Korea.

**ACKNOWLEDGEMENTS**

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