Altererythrobacter gangjinensis sp. nov., a marine bacterium isolated from a tidal flat

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A Gram-stain-negative, ochre-pigmented, strictly aerobic bacterium, designated strain KJ7\(^T\), was isolated from a tidal flat of the Gangjin bay in South Korea. Cells were halotolerant, non-motile, catalase- and oxidase-positive rods. Growth of strain KJ7\(^T\) was observed at 5–35 °C (optimum, 25 °C), at pH 6.0–9.5 (optimum, pH 6.5–7.0) and in the presence of 0–9 % (w/v) NaCl (optimum, 2 %). The major cellular fatty acids were C\(_{18:1}\)ω7C, C\(_{17:1}\)ω6c, summed feature 3 (comprising C\(_{16:1}\)ω7c and/or C\(_{16:1}\)ω6c) and C\(_{16:0}\). The polar lipid pattern indicated the presence of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, a sphenoglycolipid, an unidentified phospholipid and two unidentified lipids. The G+C content of the genomic DNA was 60.2±0.9 mol% and the predominant respiratory quinone was Q-10. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain KJ7\(^T\) formed a phyletic lineage distinct from other members of the genus Altererythrobacter and was most closely related to Altererythrobacter luteolus SW-109\(^T\) and Altererythrobacter namhicola KYW48\(^T\) (95.6 and 95.0 % 16S rRNA gene sequence similarity, respectively). On the basis of phenotypic, chemotaxonomic and molecular features, strain KJ7\(^T\) represents a novel species of the genus Altererythrobacter, for which the name Altererythrobacter gangjinensis sp. nov. is proposed. The type strain is KJ7\(^T\) (=KACC 16190\(^T\)=JCM 17802\(^T\)).

The genus Altererythrobacter, a member of the family Erythrobacteraceae of the Alphaproteobacteria, was first proposed by Kwon et al. (2007). At the time of writing, the genus Altererythrobacter contained 10 species: Altererythrobacter epoxidivorans (Kwon et al., 2007), Altererythrobacter luteolus (Yoon et al., 2005), Altererythrobacter indicus (Kumar et al., 2008), Altererythrobacter marinus (Lai et al., 2009), Altererythrobacter marenis (Seo & Lee 2010), Altererythrobacter aestivalii and Altererythrobacter namhicola (Park et al., 2011a), Altererythrobacter dongtanensis (Fan et al. 2011), Altererythrobacter xinjiangensis (Xue et al., 2012) and Altererythrobacter ishigakiensis (Matsumoto et al., 2011). All species except A. xinjiangensis were isolated from marine environments. A. xinjiangensis was isolated from a desert, but it also requires salts for growth (Xue et al., 2012), which indicates that marine environments are main habitats of members of the genus Altererythrobacter. Members of the genus are Gram-negative, aerobic, non-motile rods requiring NaCl for growth and containing C\(_{18:1}\)ω7C as the predominant fatty acid (Kwon et al., 2007). Bacteriochlorophyll a is absent in all members of the genus Altererythrobacter, while some members of the genus Erythrobacter contain bacteriochlorophyll a (Yoon et al., 2005). Sea-tidal flats are coastal marshes or muddy areas that undergo flooding with seawater and exposure to the atmosphere between low and high tides. Sea-tidal flats contain valuable biological resources such as micro-organisms and marine animals. Therefore, efforts have been made in our laboratory to isolate and characterize marine bacteria from sea-tidal flats (Jin et al., 2011a, b; Jung et al., 2011; Lee et al., 2011; Park et al., 2011b). Here, we describe the taxonomic characterization of another novel species of the genus Altererythrobacter isolated from a tidal flat of the South Sea in South Korea, for which the name Altererythrobacter gangjinensis sp. nov. is proposed.

Strain KJ7\(^T\) was isolated from the surface of a tidal flat (less than 5 cm depth) of the Gangjin bay (34° 35′ 30.31″ N 126° 46′ 12.98″ E) in South Korea using a described procedure with some modifications (Jung et al., 2011). Briefly, a tidal flat sample was serially diluted with artificial seawater (ASW; 20 g NaCl, 2.9 g MgSO\(_4\), 4.53 g MgCl\(_2\)·6H\(_2\)O, 0.64 g KCl, 1.75 g CaCl\(_2\)·2H\(_2\)O per litre), spread on marine agar 2216 (MA; Difco) and incubated at 25 °C for 5 days. Crude genomic DNA was extracted from randomly selected colonies and amplification of the 16S rRNA genes was performed using universal primers F1 (5′-AGAGTTTGTATCMTGGCTCAG-3′) and R13 (5′-TACGGYTACCTTGTTACGACTT-3′) as described by Lu et al. (2006). The amplicons were analysed on the basis of restriction

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KJ7\(^T\) is JF751048.

Three supplementary figures are available with the online version of this paper.
fragment length polymorphism after *Hae*III and *Hha*I double digestions, as described by Kim *et al.* (2010), and all representative amplicons with unique patterns were partially sequenced with the F1 primer. The resulting 16S rRNA gene sequences were analysed using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) in GenBank and the search results were used as a guide to classify the colonies. Based on the analysis, a novel strain, designated KJ7T, belonging to the genus *Altererythrobacter* was selected for further phenotypic and phylogenetic analysis. The isolate was routinely grown aerobically on MA at 25 °C for 4 days, except where indicated otherwise and stored at −80 °C in marine broth (MB) supplemented with 15% (v/v) glycerol. *A. luteolus* KCTC 12311T, *A. namihicola* KCTC 22736T and *A. epoxidivorans* JCM 13815T were used as reference strains for phenotypic characterization and fatty acid analysis.

For almost full sequencing, the 16S rRNA gene amplicon of strain KJ7T was ligated into the pCR2.1 vector using a TOPO cloning kit (Invitrogen), according to the manufacturer’s instructions. The inserted amplicon was sequenced using the M13 reverse and T7 primers of the TOPO cloning kit according to the instructions. The inserted amplicon was sequenced using the F1 primer. The resulting 16S rRNA gene sequences (1455 nt) were subsequently subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/) in GenBank and the search results were used as a guide to classify the colonies. Based on the analysis, a novel strain, designated KJ7T, belonging to the genus *Altererythrobacter* was selected for further phenotypic and phylogenetic analysis. The isolate was routinely grown aerobically on MA at 25 °C for 4 days, except where indicated otherwise and stored at −80 °C in marine broth (MB) supplemented with 15% (v/v) glycerol. *A. luteolus* KCTC 12311T, *A. namihicola* KCTC 22736T and *A. epoxidivorans* JCM 13815T were used as reference strains for phenotypic characterization and fatty acid analysis.

For almost full sequencing, the 16S rRNA gene amplicon of strain KJ7T was ligated into the pCR2.1 vector using a TOPO cloning kit (Invitrogen), according to the manufacturer’s instructions. The inserted amplicon was sequenced using the M13 reverse and T7 primers of the TOPO cloning kit. The resultant almost-complete 16S rRNA gene sequence (1455 nt) of strain KJ7T was checked manually to evaluate quality and gaps. Sequence similarity values between strain KJ7T and closely related taxa were calculated using the Nucleotide Similarity Search program (http://www.ezbiocore.co.jp/sisei/) with the EzTaxon server (Chun *et al.*, 2007) and aligned using the Greengenes alignment program (http://greengenes.lbl.gov/; DeSantis *et al.*, 2006). Phylogenetic trees using the neighbouring- and maximum-parsimony algorithms were constructed using PHYLIP version 3.6 (Felsenstein, 2002). The resulting tree topologies were evaluated using bootstrap analyses based on 1000 resampled datasets within the PHYLIP package. Maximum-likelihood analysis with bootstrap values performed using RAxML-HPC BlackBox version 7.2.8 of the Cyber-Infrastructure for Phylogenetic Research project (http://www.phylo.org/; Stamatakis *et al.*, 2005) at the San Diego Supercomputer Center.

The comparative analysis of 16S rRNA gene sequences showed that strain KJ7T was most closely related to *A. luteolus* SW-109T and *A. namihicola* KYW48T (95.6 and 95.0% sequence similarity, respectively). Phylogenetic analysis indicated that strain KJ7T formed a phyletic lineage within the genus *Altererythrobacter* (Fig. 1). Although the bootstrap value for the node between the isolate and *A. luteolus* SW-109T was relatively low (50%), the topologies of the phylogenetic trees built using the maximum-likelihood and maximum-parsimony algorithms supported the finding that strain KJ7T formed lineage separate from members of the genus *Altererythrobacter* (Fig. S1, available in IJSEM Online). Besides, a phylogenetic analysis using Ribosomal Database Project (RDP) Classifier program (Wang *et al.*, 2007) also showed that strain KJ7T represented a member of the genus *Altererythrobacter*.

Temperature and pH for growth of strain KJ7T were examined on MA at 0–45 °C (at intervals of 5 °C) and in MB at pH 4.5–10.0 (at intervals of 0.5 pH unit). The pH was adjusted prior to sterilization by the addition of HCl or NaOH and checked again after sterilization. Growth with 0–10% (w/v) NaCl (at intervals of 1%) was investigated in MB prepared in the laboratory according to the formula of the Difco medium except for the addition of NaCl. Gram-staining was performed using the bioMérieux Gram-stain kit according to the instructions of the manufacturer. Anaerobic growth was assessed on MA under anaerobic conditions (4–10% CO2) using the GasPak Plus system (BBL) at 25 °C for 4 days, except where indicated otherwise. The presence of flagella was studied using transmission electron microscopy (JEM-16101; Jeol) with 4-day-old cells grown on MA. Gliding motility was evaluated by phase-contrast microscopy (Axio Lab; Carl Zeiss) as described by Bowman (2000). The presence of flexirubin-type pigments was investigated as described elsewhere (Bernardet *et al.*, 2002; McCammon & Bowman, 2000). Bacteriochlorophyll *a* and cellular pigments were extracted in a mixture of acetone/methanol (7:2, v/v) and their absorption spectra were determined using a scanning UV/visible spectrophotometer (SynergyMx; BioTek) as described by Biebl *et al.* (2005). Oxidase activity was tested by oxidation of 1% (w/v) tetramethyl-p-phenylenediamine (Merck) and catalase activity was evaluated by the production of oxygen bubbles in 3% (v/v) aqueous hydrogen peroxide solution (Smibert & Krieg, 1994).
hydrolysis of casein, Tween 80, Tween 20, tyrosine, starch and xylan was investigated on MA according to the methods described elsewhere (Lányi, 1987; Smibert & Krieg, 1994). Voges–Proskauer test was performed as described by Smibert & Krieg (1994). Nitrate reduction was assessed according to the method of Lányi (1987). H2S production was tested as described by Bruns et al. (2001). Acid production from carbon sources was determined using the method of Leifson (1963). Utilization of various substrates for growth was determined as described by Yurkov et al. (1994). Additional enzymic activities and biochemical features of strain KJ7T and the reference strains were determined using the API ZYM and API 20 NE kits (bioMérieux), according to the manufacturer’s instructions except that inocula were prepared by suspending cells in ASW. Antibiotic susceptibility tests were performed using filter-paper discs containing the following (µg per disc unless stated otherwise): ampicillin (10), polymyxin B (100 U), streptomycin (50), penicillin G (20 U), gentamicin (30), chloramphenicol (100), tetracycline (30), kanamycin (30), lincomycin (15), oleandomycin (15), carbenicillin (100), neomycin (30) and novobiocin (5).

Table 1. Differential phenotypic characteristics of strain KJ7T and closely related members of the genus Altererythrobacter

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Cell dimensions (µm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Width</td>
<td>0.5–0.7</td>
<td>0.6–0.8</td>
<td>1.0</td>
<td>0.4–0.6</td>
</tr>
<tr>
<td>Length</td>
<td>1.5–2.8</td>
<td>1.5–3.5</td>
<td>1.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Colony colour</td>
<td>OC</td>
<td>Y</td>
<td>OR</td>
<td>Y</td>
</tr>
<tr>
<td>Methanol-soluble pigment (nm)</td>
<td>460, 473</td>
<td>332, 447, 473</td>
<td>460</td>
<td>310, 447, 473</td>
</tr>
<tr>
<td>NaCl for growth (%)</td>
<td>0–9</td>
<td>0.5–9</td>
<td>1–2</td>
<td>0.5–9</td>
</tr>
<tr>
<td>Optimum</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Temperature for growth (°C)</td>
<td>5–35</td>
<td>10–42</td>
<td>15–37</td>
<td>20–40</td>
</tr>
<tr>
<td>Optimum</td>
<td>25</td>
<td>30</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>Hydrolysis of:*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin†</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Tyrosine</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>Tween 20</td>
<td>–</td>
<td>+</td>
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</tr>
<tr>
<td>Tween 80</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activities (API ZYM)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Naphthol-AS-Bl-phosphohydrolase</td>
<td>W</td>
<td>+</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>–</td>
<td>+</td>
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<td>β-Galactosidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>60.2</td>
<td>60.3</td>
<td>63.8</td>
<td>54.5</td>
</tr>
</tbody>
</table>

*Data obtained in this study.
†This result was obtained using API 20NE.
Strain KJ7T grew at 5–35 °C, but not at 0 or 40 °C (optimum 25 °C). Cells were Gram-negative, straight, non-motile rods, 0.5–0.7 μm wide and 1.5–2.8 μm long (Fig. S2). When tested in MB at 25 °C, strain KJ7T grew at pH 6.0–9.5 (optimum pH 6.5–7.0). Strain KJ7T grew in MB containing 0.0–9.0 % (w/v) NaCl (optimum 2.0 % NaCl). Other physiological and biochemical characteristics of strain KJ7T are presented in Table 1 and the species description. Some of them are in accordance with characteristics of the reference strains, whereas others allow the differentiation of strain KJ7T from its closest relatives (Table 1).

Isoprenoid quinones were analysed using HPLC (model LC-20A; Shimadzu) equipped with a diode array detector (SPD-M20A; Shimadzu) and a reversed-phase column (250 × 4.6 mm, Kromasil; Akzo Nobel) as described by Komagata & Suzuki (1987). For analysis of cellular fatty acids, strain KJ7T and the reference strains were cultivated in Komagata & Suzuki (1987). For analysis of cellular fatty acids, strain KJ7T and the reference strains were cultivated in MB at 25 °C and cells were harvested at the range for the genus Altererythrobacter content of strain KJ7T was 60.2 ± 0.9 mol%, which is within the range for the genus Altererythrobacter (54.5–67.2 mol%).

The polar lipid profile consisted of phosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and a sphingoglycolipid as the major components. Diphosphatidylglycerol, two unidentified lipids and an unidentified phospholipid were also identified as minor components (Fig. S3). The major cellular fatty acids (>5 % of the total fatty acids) were C18:1ω7c (47.9 %), C17:0ω6c (16.2 %), summed feature 3 (comprising C16:1ω7c and/or C16:1ω6c 11.2 %) and C16:0 (6.3 %). Although the overall fatty acid profile of strain KJ7T was similar to those of closely related members within the genus Altererythrobacter, the proportions and presence or absence of some fatty acids distinguished the isolate from the reference strains (Table 2). The G+C content of strain KJ7T was 60.2 ± 0.9 mol%, which is within the range for the genus Altererythrobacter (54.5–67.2 mol%).

Therefore, on the basis of the physiological, biochemical and phylogenetic properties described above, strain KJ7T should be placed into a novel species within the genus Altererythrobacter, for which the name Altererythrobacter gangjinensis sp. nov. is proposed.

### Table 2. Whole-cell fatty acid content (%) of strain KJ7T and closely related members of the genus Altererythrobacter

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>6.3</td>
<td>6.8</td>
<td>9.0</td>
<td>4.8</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.7</td>
<td>2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsaturated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:1ω5c</td>
<td>1.0</td>
<td>2.5</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>C17:1ω6c</td>
<td>16.2</td>
<td>21.3</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>C17:1ω8c</td>
<td>4.0</td>
<td>3.1</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>C18:1ω5c</td>
<td>1.0</td>
<td>1.2</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>C18:1ω7c</td>
<td>47.9</td>
<td>51.6</td>
<td>32.8</td>
<td>51.7</td>
</tr>
<tr>
<td>11-Methyl-C18:1ω7c</td>
<td>4.7</td>
<td>5.4</td>
<td></td>
<td>4.1</td>
</tr>
<tr>
<td>Hydroxy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0 2-OH</td>
<td>1.2</td>
<td>2.6</td>
<td>2.9</td>
<td>6.6</td>
</tr>
<tr>
<td>C15:0 2-OH</td>
<td>1.2</td>
<td>2.0</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>C16:0 2-OH</td>
<td>1.0</td>
<td>1.4</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>C16:1 3-OH</td>
<td>2.2</td>
<td>1.2</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>iso-C16:0 3-OH</td>
<td></td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Summed feature 3</td>
<td>11.2</td>
<td>25.1</td>
<td>23.0</td>
<td>22.4</td>
</tr>
</tbody>
</table>

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of C16:1ω7c and/or C16:1ω6c.

The species description. Strains: 1, strain KJ7T; 2, A. luteolus KCTC 12311T; 3, A. namnicola KCTC 22736T; 4, A. epoxidovans JCM 13815T. All data obtained in this study.

Cells are Gram-stain-negative, chemoheterotrophic, aerobic, non-spore-forming rods (0.5–0.7 × 1.5–2.8 μm) and show no flagellar or gliding motility. Colonies are ochre, convex and round on MA. Growth occurs at 5–35 °C (optimum, 25 °C), at pH 6.0–9.5 (optimum, pH 6.5–7.0) and in the presence of 0.0–9.0 % (w/v) NaCl (optimum 2.0 % NaCl). Anaerobic growth is not observed after 20 days at 25 °C on MA. Flexirubin-type pigments are absent. Does not contain bacteriochlorophyll a as a photosynthetic pigment. Methanol-soluble pigment is characterized by two absorption maxima at 460 and 473 nm. Oxidase- and catalase-positive. Tween 80 is hydrolysed, while casein, Tween 20, starch, tyrosine and xylan are not. H2S is not produced. Voges–Proskauer reaction, nitrate reduction and denitrification are negative. In API 20NE tests, β-galactosidase activity, hydrolysis of aesculin and assimilation of D-mannitol are positive, but indole production, glucose fermentation, arginine dihydrolase activity, urease activity, hydrolysis of gelatin and assimilation of N-acetylglucosamine, adipate, L-arabinose, caprate, trisodium citrate, potassium gluconate, D-glucose, malate, malteose, D-mannose and phenylacetate are negative. In API ZYM tests, alkaline phosphatase, esterase (C4), lipase (C8), valine arylamidase, leucine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase and β-galactosidase activities are positive, naphthol-AS-Bl-phosphohydrolase activity is weakly positive, but esterase (C4),...
lipase (C14), α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-manno-
sidase and α-fucosidase activities are negative. D-Glucose is weakly utilized as a sole carbon and energy source, but D-fructose, D-galactose, sucrose, lactose, D-mannose, D-, L-
malic acid, citric acid, melibiose, raffinose, D-sorbitol and D-mannitol are not utilized. Acid is not produced from
D-glucose, D-fructose, lactose, D-galactose, D-mannose, melibiose, raffinose, sucrose, D-sorbitol or D-mannitol.
Phosphatidylycerine, phosphatidylethanolamine, phosphatidyglycerol and a sphingolipid are the main polar
lipids. Strain KJ7T is resistant to streptomycin, ampicillin, gentamicin, tetracycline and lincomycin, but sensitive to
dopolymixin B, penicillin G, chloramphenicol, kanamycin, novobiocin, oleandomycin, neomycin and carbenicillin.
The major cellular fatty acids (>5% of the total fatty acids) are C18:1ω7c, C17:1ω6c summed feature 3 (comprising
C16:1ω7c and/or C16:1ω6c) and C16:0.
The type strain is KJ7T (=KACC 16190T=JCM 17802T),
which was isolated from a tidal flat of the Gangjin bay in
South Korea. The DNA G+C content of the type strain is
60.2 ± 0.9 mol%.

Acknowledgements
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Forestry and Fisheries and by the Next-Generation BioGreen 21
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Korea (SSAC2011- P008220).

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University of Washington, Seattle, USA.
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