Sphingomonas aerophila sp. nov. and Sphingomonas naasensis sp. nov., isolated from air and soil, respectively

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Two strains, designated 5413J-26T and KIS18-15T, were isolated from the air and forest soil, respectively, in South Korea. Cells of the two strains were Gram-stain-negative, aerobic, polar-flagellated and rod-shaped. According to the phylogenetic tree, strains 5413J-26T and KIS18-15T fell into the cluster of Sphingomonas sensu stricto. Strain 5413J-26T showed the highest sequence similarities with Sphingomonas trueperi LMG 2142T (96.6 %), Sphingomonas molluscorum KMM 3882T (96.5 %), Sphingomonas azotifigens NBRC 15497T (96.3 %) and Sphingomonas pituitosa EDIVT (96.1 %), while strain KIS18-15T had the highest sequence similarity with Sphingomonas soli T5-04T (96.8 %), Sphingomonas pituitosa EDIVT (96.6 %), Sphingomonas leidyi ATCC 15260T (96.6 %), Sphingomonas asaccharolytica NBRC 15499T (96.6 %) and Sphingomonas koreensis JSS26T (96.6 %). The 16S rRNA gene sequence similarity between strains 5413J-26T and KIS18-15T was 95.4 %. Ubiquinone 10 was the predominant respiratory quinone and homospermidine was the major polyamine. The major polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and several unidentified phospholipids and lipids. The main cellular fatty acids (>10 % of the total fatty acids) of strain 5413J-26T were summed feature 8 (C18 : 1ω6c and/or C18 : 1ω7c), summed feature 3 (C16 : 1ω7c and/or iso-C15 : 02-OH) and C14 : 0 2-OH, and those of strain KIS18-15T were summed feature 8 and C16 : 0. Based on the results of 16S rRNA gene sequence analysis, and physiological and biochemical characterization, two novel species with the suggested names Sphingomonas aerophila sp. nov. (type strain 5413J-26T=KACC 16533T=NBRC 108942T) and Sphingomonas naasensis sp. nov. (type strain KIS18-15T=KACC 16534T=NBRC 108943T) are proposed.

The genus Sphingomonas was classified into the family Sphingomonadaceae, the order Sphingomonadales and the α-4 group of the class Alphaproteobacteria. The genus Sphingomonas was first proposed by Yabuuchi et al. (1990) for Gram-negative, strictly aerobic, chemoheterotrophic, yellow and rod-shaped bacteria with sphingoglycolipids as polar lipids. The genus Sphingomonas was reclassified into four groups on the basis of phylogenetic, chemotaxonomic and phenotypic data: Sphingomonas sensu stricto and three new genera, Sphingobium, Novosphingobium and Sphingopyxis (Takeuchi et al., 2001). Recently, Caulobacter leidyi was reclassified as Sphingomonas leidyi (Chen et al., 2012). However, Yabuuchi et al. (2002) suggested that there was no phenotypic and phylogenetic evidence to divide the genus Sphingomonas into the four separate genera, and that all four genera and another closely related genus, Blastomonas, should remain as one genus, Sphingomonas. At present, all four genus names except for the genus Blastomonas are still used. Species of the genus Sphingomonas are widely distributed in soil, water, clinical specimens, the plant phyllosphere and rhizosphere and other locations.

Strain 5413J-26T was isolated from outside air in Jeju Island, South Korea. An MAS-100 air sampler (single-stage multiple-hole impactor; Merck) containing a Petri dish with R2A agar (BBL) amended with 200 μg cycloheximide ml⁻¹ (Sigma) was used to trap an air sample outside. Strain
KIS18-15T was isolated from forest soil collected from Baengnyeong Island, South Korea. Soil samples were serially diluted with 0.85 % (w/v) saline, and the suspensions were plated on R2A agar (Difco).

Genomic DNA was extracted using the method of Ausubel et al. (1987). The 16S rRNA gene was amplified using the universal primers 9F and 1512R (Weisburg et al., 1991), and the purified PCR products were sequenced by Solgent (Daejeon, South Korea). The PCR product was purified using a PCR purification kit (Qiagen). Sequencing of the 16S rRNA gene was carried out at Solgent (Daejeon, South Korea) with an Applied Biosystems automatic sequencer using the sequencing primers 27F (5' AGAGTTTGATCCTGGCTCAG-3'), 1492R (5'-GGCTACCTTGTTACGACTT-3'), 1581R (5'-GTATTACCGGGCTGTGAG-3') and 785F (5'-GGATTAGATACCCTGTA-3') to generate a consensus sequence. SeqMan software (DNASTAR) was used to assemble almost full-length 16S rRNA gene sequences. The EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012) was used to search for phylogenetic neighbors based on 16S rRNA gene sequence similarities. Alignment and analysis of sequence data were performed using the ARB software package (version December 2007; Ludwig et al., 2004) and the corresponding SILVA SSURef 100 database (released August 2009; Pruesse et al., 2007). Phylogenetic trees were reconstructed using MEGA5.0 (Tamura et al., 2011) on the basis of the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) algorithms. The 16S rRNA gene sequence lengths of strains 5413J-26T and KIS18-15T were 1428 and 1426 bp, respectively. The level of sequence similarity between strains 5413J-26T and KIS18-15T was 95.4 %. Strain 5413J-26T showed the highest sequence similarities with Sphingomonas trueperi LMG 2142T (96.6 %), Sphingomonas molluscorum KMM 3882T (96.5 %), Sphingomonas azotifigens NBRC 15497T (96.3 %) and Sphingomonas pituitosa EDIVT (96.1 %), with less than 96 % sequence similarities with other species of the genus Sphingomonas. The 16S rRNA gene sequence of strain KIS18-15T had the highest sequence similarity with Sphingomonas soli T5-04T (96.8 %), Sphingomonas pituitosa EDIVT (96.6 %), Sphingomonas leidyi ATCC 15260T (96.6 %), Sphingomonas asaccharolytica NBRC 15499T (96.6 %) and Sphingomonas koreensis JSS26T (96.6 %), with below 96.5 % sequence similarity with other species of the genus Sphingomonas. According to the phylogenetic tree (Fig. 1), strains 5413J-26T and KIS18-15T fell into the cluster of Sphingomonas sensu stricto (Takeuchi et al., 2001). The positioning of strains 5413J-26T and KIS18-15T within the genus Sphingomonas sensu stricto was also supported by the maximum-parsimony and maximum likelihood trees (Fig. 1).

To observe cell morphology, strains 5413J-26T and KIS18-15T were grown on R2A agar at 28 °C for 2 days and viewed under a light microscope (AXIO; Zeiss) and by transmission electron microscopy (model 912AB; LEO). Growth of the novel strains on Luria–Bertani (LB) agar, nutrient agar (NA), tryptone soya agar (TSA) and MacConkey agar (all from Difco) was checked. Anaerobic growth was determined from incubation in the BBL GasPak Anaerobic System (Difco) for 14 days at 28 °C on R2A agar. Gram staining was determined using a Difco Gram staining kit, according to the manufacturer’s instructions. Catalase and oxidase activities were examined by bubble production in 3 % (v/v) hydrogen peroxide solution and 1 % (w/v) tetramethyl-p-phenylenediamine, respectively. Growth was examined at 4, 10, 15, 20, 25, 28, 30, 37, 40 and 45 °C and at pH 4.0–11.0 (at intervals of 1.0 pH unit), adjusted with 0.2 M citrate/phosphate buffer, 0.05 M Tris/HCl buffer, HCl or NaOH (Breznak & Costilow, 1994) on R2A broth. NaCl tolerance (up to 5 %; w/v) was tested in R2A broth. Casein, starch and tyrosine degradations were tested on R2A plates containing milk powder (5 %, w/v), starch (1 %, w/v) and tyrosine (0.1 %, w/v), respectively. CM-cellulose and Tween 80 degradation were examined using R2A supplemented with 1 % (w/v) of each substrate. DNase activity was determined with DNase test agar (Difco). Other physiological properties, utilization of various carbon sources and enzyme activity were tested in duplicate with commercial API 20NE, API ID 32GN and API ZYM kits (bioMérieux), according to the protocols provided by the manufacturers. API 20NE, and API ID 32GN test strips were checked after 5 days of incubation, and API ZYM kits were tested after incubation of 4 h at 28 °C. Both strains were Gram-stain-negative, aerobic, polar-flagellated rods (Fig. S1, available in the online supplementary material). Both strains grew on R2A, LB, NA and TSA; however, not on MacConkey agar. Strain 5413J-26T grew in the temperature range 4–37 °C (optimum 28–30 °C) and pH 6–9 (optimum pH 7.0) and tolerated up to 1 % (w/v) NaCl (optimum 0 %), while strain KIS18-15T grew at 10–37 °C (optimum, 28–30 °C), pH 6–9 (optimum, pH 7.0) and 0–0.5 % (w/v) NaCl (optimum, 0 %). The detailed phenotypic characterization of strains 5413J-26T and KIS18-15T is given in the species descriptions and presented in Table 1.

Quinones and polar lipids were extracted and analysed according to the protocol of Minnikin et al. (1984). For cellular fatty acid analysis, all strains were cultured in R2A at 28 °C for 48 h when all of the tested strains were in the exponential phase. The cellular fatty acids were extracted, methylated and separated by gas chromatography (model 6890; Hewlett Packard) according to the protocol of the Sherlock Microbial Identification System (MIDI; Sasser, 1990) version 6.10, with database TSBA 6 used to assign GC peaks to each fatty acid. Polyamines were analysed by HPLC (LC-10A; Shimadzu), with the chromatogram equipped with a fluorescence detector (RF-10AXL; Shimadzu) and a reversed-phase column (Kromasil ODS; 250x6.6 mm; Akzo Nobel) as described previously (Busse & Aulig, 1988; Busse et al., 1997). Strains 5413J-26T and KIS18-15T contained 89.7 and 83.6 %, respectively, ubiquinone 10 (Q-10) as the predominant respiratory quinone and small amounts of Q-8 (3.3 and 4.7 %, respectively), Q-9 (3.9 and 4.8 %, respectively) and Q-11 (3.2 and...
6.8 %, respectively), which is in congruence with previous findings (Yabuuchi et al., 1990; Rivas et al., 2004; Huang et al., 2012). The polar lipids of strain 5413J-26T consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, three unidentified phospholipids and two unidentified lipids (Fig. S2). The polar lipids of strain KIS18-15T were more complex than those of strain 5413J-26T, and were composed of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, three unidentified phospholipids and four unidentified lipids (Fig. S3). However, the polar lipid profiles of both strains lacked sphingoglycolipids, which are characteristic of species of the genus Sphingomonas (Chen et al., 2012; Wittich et al., 2007; Yabuuchi & Kosako, 2005). The fatty acid compositions of strains 5413J-26T and KIS18-15T are shown in Table 2. The fatty acid profiles of both strains lacked sphingoglycolipids, which is present in all members of the genus Sphingomonas (Yabuuchi et al., 1990; Rivas et al., 2004; Huang et al., 2013). The polar lipids of strain 5413J-26T consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, three unidentified phospholipids and four unidentified lipids (Fig. S2). The polar lipids of strain KIS18-15T were more complex than those of strain 5413J-26T, and were composed of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, three unidentified phospholipids and four unidentified lipids (Fig. S3). However, the polar lipid profiles of both strains lacked sphingoglycolipids, which are characteristic of species of the genus Sphingomonas (Chen et al., 2012; Wittich et al., 2007; Yabuuchi & Kosako, 2005). The fatty acid compositions of strains 5413J-26T and KIS18-15T are shown in Table 2. The fatty acid composition of species of the genus Sphingomonas are highly variable with respect to the relative amounts of fatty acids such as C17:1ω6c, C17:1ω8c and summed features (An et al., 2013; Chung et al., 2011). Among the strains tested, strains 5413J-26T, KIS18-15T, Sphingomonas paucimobilis KACC 1093T and Sphingomonas trueperi KACC 12329T showed similar fatty acid profiles with small amounts of C17:1ω6c and C17:1ω8c, compared to that of Sphingomonas soli KACC 13009T (Table 2). Sphingomonas soli KACC 13009T had a fatty acid composition similar to that of Sphingomonas jaspsi and Sphingomonas sediminicola (An et al., 2013). The major polyamine of both the novel strains was homospermidine, which is present in all members of the genus Sphingomonas with validly published names (Maruyama et al., 2006; Takeuchi et al., 2001; Hamana et al., 2003). The polyamine pattern of strain 5413J-26T demonstrated the presence of a large amount of homospermidine [38.3 μmol (g dry weight)^−1] with minor amounts of spermidine [2.8 μmol (g dry weight)^−1], putrescine [1.1 μmol (g dry weight)^−1] and spermine [0.7 μmol (g dry weight)^−1], and that of strain KIS18-15T a large amount of homospermidine [20.6 μmol (g dry weight)^−1] with minor amounts of 2-hydroxy putrescine [3.6 μmol (g dry weight)^−1], putrescine [1.1 μmol (g dry weight)^−1] and 1,3-diaminopropane [0.5 μmol (g dry weight)^−1].
On the basis of phylogenetic analysis (Fig. 1), physiological and biochemical characteristics (Table 1), fatty acid profiles (Table 2), polar lipids (Figs. S2 and S3) and patterns of polyamines, we suggest that strains 5413J-26T and KIS18-15T represent two novel species of the genus *Sphingomonas*, for which the names *Sphingomonas aerophila* sp. nov.
Table 2. Fatty acid comparison between strain 5413J-26T, strain KIS18-15T and type strains of closely related species of the genus Sphingomonas

Strains: 1, 5413J-26T; 2, KIS18-15T; 3, Sphingomonas paucimobilis KACC 10931T; 4, Sphingomonas soli KACC 13009T; 5, Sphingomonas truoperi KACC 12329T. All data were obtained in this study. All strains were grown on R2A at 28 °C for 48 h. Fatty acids amounting to <0.5% of total fatty acids are not shown.

<table>
<thead>
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<th>Fatty acid</th>
<th>1</th>
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<th>3</th>
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<tr>
<td>C14:0 2-OH</td>
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<td>8.4</td>
<td>5.9</td>
<td>2.6</td>
<td>5.9</td>
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<tr>
<td>C15:0 2-OH</td>
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<td>0.6</td>
<td>–</td>
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<tr>
<td>C16:0</td>
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<tr>
<td>C16:0 2-OH</td>
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<td>1.1</td>
<td>–</td>
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<tr>
<td>C16:1ω5c</td>
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<td>0.9</td>
<td>0.7</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>C17:0</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>1.1</td>
</tr>
<tr>
<td>C17:0 Cyclo</td>
<td>–</td>
<td>–</td>
<td>1.9</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C18:1ω6c</td>
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<tr>
<td>C18:1ω8c</td>
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<tr>
<td>C18:1ω5c</td>
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<td>C18:1ω7c 11-methyl</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>C18:1 2-OH</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>C19:0 Cyclo ω8c</td>
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<td>–</td>
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<td>2.1</td>
<td>1.9</td>
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<td>67.9</td>
<td>41.2</td>
<td>75.4</td>
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</table>

*Summed feature 3 contains C16:1ω7c and/or iso-C15:0 2-OH. Summed Feature 8 contains C18:1ω6c and/or C18:1ω7c.

and Sphingomonas naasensis sp. nov., respectively, are proposed.

Description of Sphingomonas aerophila sp. nov.

Sphingomonas aerophila [ae.ro’phi.la. Gr. masc. n. aer air; N.L. adj. philus -a -um (from Gr. adj. philos -ē -on) friend, loving; N.L. fem. adj. aerophila air-loving].

Gram-stain-negative, aerobic, polar-flagellated and rod-shaped (0.4–0.5 μm × 1.0–2.0 μm). After incubation for 2 days on R2A agar colonies are light-yellow, circular, convex with regular edges. Oxidase-positive and catalase-negative. Growth occurs at temperatures of 10–37 °C (optimum 28–30 °C). Growth occurs at pH 6.0–9.0, (optimum at pH 7.0). Up to 0.5 % (w/v) NaCl is tolerated. Cellulose, Tween 80 and tyrosine are hydrolysed, but not casein, chitin, DNA, hypoxanthine, starch or xanthine. Positive for aesculin hydrolysis and β-galactosidase (PNG), but negative for arginine dihydrolase, gelatin hydrolysis, glucose fermentation, indole production, nitrate reduction and urease (API 20NE test strips). Assimilates N-acetylglucosamine, L-arabinose, L-ribose, D-glucose, glycogen, L-histidine, 3-hydroxybutyric acid, maltose, D-mannose and L-rhamnose, and weakly melibiose, sucrose and salicin, but does not assimilate adipic acid, L-alanine, capric acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid inositol, itaconic acid, lactic acid, malic acid, d-mannitol, phenylacetic acid, potassium 2-ketogluconate, potassium 5-ketogluconate, L-proline, D-ribose, sucrose, L-serine, sodium acetate, sodium malonate, D-sorbitol, suberic acid, trisodium citrate or valeric acid (API 20NE and API ID 32GN test strips). Positive for N-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C8), β-galactosidase, α-glucosidase, β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase activity, but negative for α-chymotrypsin, α-fucosidase, lipase (C14), α-mannosidase and trypsin (API ZYM test strips) activity. The predominant respiratory quinone is Q-10, the major polyamine is homospermidine, and the main cellular fatty acids are summed feature 8 (C18:1ω6c and/or C18:1ω7c), summed feature 3 (C16:1ω5c and/or iso-C15:0 2-OH) and C14:0 2-OH. The polar lipids present are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, three unidentified phospholipids and two unidentified lipids.

The type strain, 5413J-26T (=KACC 16533T=NBRC 108942T), was isolated from outside air in Jeju Island, South Korea.

Description of Sphingomonas naasensis sp. nov.

Sphingomonas naasensis (naa.sen’sis. N.L. fem. adj. naasensis pertaining to NAAS, the acronym for the National Academy of Agricultural Science, where the taxonomic studies on the type strain were first performed).

Gram-stain-negative, aerobic, polar-flagellated and rod-shaped (0.4–0.5 μm × 1.0–1.4 μm). After incubation for 2 days on R2A agar colonies are orange, smooth, shiny and opaque with regular edges. Oxidase-positive and catalase-negative. Growth occurs at temperatures of 4–37 °C and optimally at 28–30 °C. Growth occurs at pH 6.0–9.0, (optimum at pH 7.0). Up to 0.5 % (w/v) NaCl is tolerated. Cellulose, Tween 80 and tyrosine are hydrolysed, but not casein, chitin, DNA, hypoxanthine, starch or xanthine. Positive for aesculin hydrolysis and β-galactosidase (PNG), but negative for arginine dihydrolase, gelatin hydrolysis, glucose fermentation, indole production, nitrate reduction and urease (API 20NE test strips). Assimilates N-acetylglucosamine, L-arabinose, L-ribose, D-glucose, glycogen, L-histidine, 3-hydroxybutyric acid, maltose, D-mannose and L-rhamnose, and weakly melibiose, sucrose and salicin, but does not assimilate adipic acid, L-alanine, capric acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid inositol, itaconic acid, lactic acid, malic acid, D-mannitol, phenylacetic acid, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, L-proline, propionic acid, D-ribose, L-serine, sodium acetate, sodium malonate, D-sorbitol, suberic acid, trisodium citrate or valeric acid (API 20NE and API ID 32GN test strips). Positive for N-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C8), β-galactosidase, α-glucosidase, β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase activity, but negative for α-chymotrypsin, α-fucosidase, lipase (C14), α-mannosidase and trypsin (API ZYM test strips) activity. The predominant respiratory quinone is Q-10, the major polyamine is homospermidine, and the main cellular fatty acids are summed feature 8 (C18:1ω6c and/or C18:1ω7c), summed feature 3 (C16:1ω5c and/or iso-C15:0 2-OH) and C14:0 2-OH. The polar lipids present are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, three unidentified phospholipids and two unidentified lipids.

The type strain, 5413J-26T (KACC 16533T=NBRC 108942T), was isolated from outside air in Jeju Island, South Korea.
arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase activity, and weakly positive for esterase (C4), but negative for α-chymotrypsin, α-fucosidase, α-galactosidase, β-glucuronidase, lipase (C14), α-mannosidase and trypsin (API ZYM) activity. The predominant respiratory quinone is Q-10, the major polyamine is homospermidine, and the main cellular fatty acids are summed feature 8 (C18:1ω6c and/or C18:1ω7c) and C16:0. The polar lipids present are diphosphatidylglycerol, phosphatidylglycerol, phosphatidyl ethanolamine, phosphatidylmonomethylthanolamine, an unidentified aminophospholipid, five unidentified phospholipids, and four unidentified lipids.

The type strain, KIS18-15T (=KACC 16534T=NBRC 108943T), was isolated from forest soil from Baengnyeong Island, South Korea.

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References


