The order *Oceanospirillales* within the class *Gammaproteobacteria* (Garrity et al., 2005a) is largely based on 16S rRNA gene sequence phylogeny. The order encompasses a diverse range of Gram-negative bacteria that are generally halophilic or halotolerant, rod-shaped, motile (except for the members of the genus *Alcanivorax*) and chemoheterotrophic. The order currently contains five families with validly published names and one that remains to be validly published: *Oceanospirillaceae* (Garrity et al., 2005b), *Alcanivoracaceae* (Golyshin et al., 2005), *Halomonadaceae* (Franzmann et al., 1988), *Hahellaceae* (Garry et al., 2005c), *Oleiphilaceae* (Golyshin et al., 2002) and ‘*Saccharosporillaceae*’ (Labrenz et al., 2003). In this study, we report on the isolation and taxonomy of a single strain that could not be assigned to any of the defined families in the order *Oceanospirillales*.

A sample of coastal seawater was collected, at a depth of 10 m, near Goseong, East Sea, Korea (38° 20’ N 128° 33’ E), in June 2005. An aliquot (100 µl) of the seawater sample was spread onto an oligotrophic medium, R2A agar (Difco) diluted 1:10 (v/v) with aged seawater (referred to as 1/10R2A), and the agar plates were incubated aerobically at 20 °C for 1 month. Strain IMCC1097<sup>T</sup>, initially grown on 1/10R2A, was further purified on marine agar 2216 (MA; Difco) after growth of the strain at 20 °C for 2 weeks. After the optimum growth temperature for the strain had been determined, cultures were maintained routinely on MA at 25 °C.

The almost-complete sequence of the 16S rRNA gene (1483 bp) for strain IMCC1097<sup>T</sup> was obtained as described previously (Cho & Giovannoni, 2003). Phylogenetic analyses, including multiple alignment of 16S rRNA gene sequences and generation of phylogenetic trees, were performed using the ARB package (Ludwig et al., 2004) and PAUP* (Swofford, 2002) as described by Cho & Giovannoni (2006). Preliminary sequence comparisons against the 16S rRNA gene sequences deposited in the GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMCC1097<sup>T</sup> is EF176580.

Transmission electron micrographs of cells of strain IMCC1097<sup>T</sup> are available as a supplementary figure with the online version of this paper.

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**Litoricolaceae fam. nov., to include *Litoricola lipolytica* gen. nov., sp. nov., a marine bacterium belonging to the order *Oceanospirillales***

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Division of Biology and Ocean Sciences, Inha University, Namgu, Incheon 402-751, Republic of Korea

A Gram-negative, non-motile, chemoheterotrophic, facultatively aerobic, short-rod-shaped bacterium, designated IMCC1097<sup>T</sup>, was isolated from coastal seawater (10 m depth) of the East Sea, Korea. The temperature, pH and NaCl ranges for growth were 15–30 °C, pH 5.0–10.0 and 1.5–10 % NaCl. The colonies of the strain were very small, having a mean diameter of 0.05 mm. 16S rRNA gene sequence data indicated that the strain was most closely related to genera within the class *Gammaproteobacteria*. Members of the most closely related genera showed less than 90 % sequence similarity and included *Saccharosporillum* (89.3 %), *Oleiphilus* (88.7 %), *Reinekea* (88.2 %), *Alcanivorax* (86.4–87.6 %) and *Zooshikella* (87.6 %), which represent five different families of the order *Oceanospirillales*. Phylogenetic analyses showed that this marine strain represented a distinct phylogenetic lineage in the order *Oceanospirillales* and could not be assigned to any of the defined families in the order. The predominant fatty acids were C<sub>16:0</sub> 10c and/or iso-C<sub>15:0</sub> 2-0H, C<sub>18:1ω7c</sub> and C<sub>10:0</sub> 3-0H, and the DNA G + C content was 57.9 mol%. These chemotaxonomic properties, together with phenotypic characteristics, served to differentiate the strain from phylogenetically closely related genera. The very low sequence similarities (<90 %) and distant relationships between IMCC1097<sup>T</sup> and members of the order *Oceanospirillales* suggested that the strain merited classification within a novel genus within a novel family in the order. On the basis of taxonomic evidence collected in this study, a novel genus and species are proposed, *Litoricola lipolytica* gen. nov., sp. nov., within a new family *Litoricolaceae* fam. nov. Strain IMCC1097<sup>T</sup> (=KCCM 42360<sup>T</sup> =NBRC 102074<sup>T</sup>) is the type strain of *Litoricola lipolytica*.  

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMCC1097<sup>T</sup> is EF176580.
GenBank and the Ribosomal Database Project showed that the strain belonged to the class Gammaproteobacteria. The sequence similarity of strain IMCC1097T with respect to recognized species within the Gammaproteobacteria was very low: no species with validly published names showed more than 90% sequence similarity. Comparative analyses of 16S rRNA gene sequence similarity based on manually aligned sequences in the ARB database showed that the most closely related type strains of species with validly published names included Saccharosporillum impatiens DSM 12546T (89.3%), Oleiphilus messinensis DSM 13489T (88.7%), Reinekea marin sedimentorum DSM 15388T (88.2%), Alcanivorax borkumensis ATCC 700651T (87.6%) and Zooshikella ganghwensis DSM 15267T (87.6%). The above species all belong to the order Oceanospirillales; however, they represent five different families in the order, which indicates a unique phylogeny for strain IMCC1097T. In all of the phylogenetic trees, generated using three different algorithms (Fig. 1), strain IMCC1097T formed a robust monophyletic clade with uncultured bacteria F3C13 (99.5% sequence similarity; Prabagaran et al., 2007) and CHAB-V-35 (97.6% sequence similarity; Schäfer et al., 2000). This monophyletic clade containing the novel isolate was clearly distinguishable from other families within the order Oceanospirillales. In the maximum-likelihood and neighbour-joining trees, the clade containing strain IMCC1097T formed a larger clade with Z. ganghwensis DSM 15267T (Fig. 1). However, this relationship between IMCC1097T and Z. ganghwensis was not found in the maximum-parsimony tree, and moreover, the bootstrap percentages obtained did not support monophyletic relationships for the clade. According to our phylogenetic analyses, the order Oceanospirillales and the families Oceanospirillaceae and Hahellaceae had polyphyletic properties. In spite of incomplete phylogenetic resolution of the order Oceanospirillales and the class Gammaproteobacteria, the very low sequence similarities (<90%) and the distant relationships between strain IMCC1097T and other families within the order Oceanospirillales suggested that the strain represented a novel genus within a novel family in the order Oceanospirillales.

Phenotypic and chemotaxonomic characteristics were determined using MA at 25°C, unless otherwise indicated. Cellular morphology was examined with transmission electron microscopy (CM200; Philips). Exponentially grown bacterial cultures were washed with sodium cacodylate buffer twice and negatively stained with 2% phosphotungstic acid (pH 7.0–7.2) on Formvar-coated copper grids. Cell size and morphology were also determined using phase-contrast microscopy and epifluorescence microscopy (Nikon 80i) with 4',6-diamidino-2-phenylindole (DAPI) staining. Motility was tested from wet mounts of exponential-phase cells. The presence of poly-β-hydroxybutyrate granules was checked using epifluorescence microscopy after staining of the cells with Nile blue A (Ostle & Holt, 1982). Colony morphology, size and colour were examined using cultures grown aerobically on MA for 1 week. The capacity of strain IMCC1097T for anaerobic growth was tested using the MGC anaerobic system with AnaeroPACK Anaero (Mitsubishi Gas Chemical) with cells incubated for up to 3 weeks. A catalase test was performed by adding 3.0% hydrogen peroxide to fresh colonies, and oxidase activity was determined using Kovács solution (Kovács, 1956). The temperature range and optimum were tested from 4 to 42°C. The pH range and optimum were examined from pH 4.0 to 12.0. The NaCl concentrations and optimum for growth were determined in NaCl-free artificial seawater medium (ASW; basic formula, with NaCl, containing the following, l−1: 19.45 g NaCl, 5.9 g MgCl2·6H2O, 3.24 g MgSO4·7H2O, 1.8 g CaCl2·2H2O, 0.55 g KCl, 0.16 g NaHCO3, 0.08 g KBr, 0.38 g SrCl2·6H2O, 0.079 g BaCl2·2H2O, 0.0074 g Na2HPO4, 0.004 g Na2SiO3, 0.0024 g NaF, 0.0016 g KNO3), supplemented with 5.0 g peptone, 1.0 g yeast extract and 0–15% (w/v) NaCl. Other biochemical tests were performed using API 20NE and API ZYM (bioMérieux) according to the manufacturer’s instructions, by inoculating the cells into ASW medium. The utilization of various compounds as sole carbon sources was tested as described in a previous study (Cho & Giovannoni, 2003), using custom-made 48-well microtitre plates containing 47 different carbon compounds (listed in the species description) at a final concentration of 0.02% (w/v or v/v) in ASW medium. The microtitre plates were incubated aerobically at 25°C for 1 week, and cellular growth in each well was screened using epifluorescence microscopy with DAPI staining. Susceptibility to the following antimicrobial agents was determined (using the diffusion plate method): tetracycline, 30 μg; ampicillin, 10 μg; kanamycin, 30 μg; chloramphenicol, 25 μg; erythromycin, 15 μg; gentamicin, 10 μg; penicillin G, 10 μg; streptomycin, 10 μg; vancomycin, 30 μg; and rifampicin, 50 μg. The DNA G+C content of strain IMCC1097T was analysed by using HPLC according to Mesbah et al. (1989). Cellular fatty acid methyl esters were prepared from cultures grown on MA at 25°C for 1 week, and analysed according to the instructions of the Microbial Identification System (MIDI) by the Korean Culture Center of Microorganisms.

Cells of strain IMCC1097T were Gram-negative, non-pigmented, chemoheterotrophic, non-motile, facultatively aerobic, short rods that required NaCl for growth. The colonies were 0.05 mm in diameter, increasing to 1 mm after a prolonged incubation period of 3 weeks. The taxonomic characteristics of the strain are described in detail in the genus and species descriptions. Several phenotypic and genomic characteristics clearly differentiated strain IMCC1097T from phylogenetically distantly related genera in the families Hahellaceae, Oceanospirillaceae, Oleiphilaceae, ‘Saccharosporillaceae’ and Alcanivoraceae (Table 1). The strain was differentiated from the genera Zooshikella and Hahella on the basis of several characteristics, including pigmentation, flagellar motility, catalase activity and DNA G+C content. The major cellular fatty acids detected in strain IMCC1097T were...
C16:1ω7c and/or iso-C15:0 2-OH (42.8 %), C18:1ω7c (20.6 %), C10:0 3-OH (14.1 %) and C16:0 (6.5 %), and the overall fatty acid composition was different from that of other genera of the order Oceanospirillales (Table 2). The presence of C10:0 3-OH and C12:1 3-OH and the absence of C18:1ω9c in IMCC1097T could serve as a fatty acid signature for differentiating the strain from the members of the genera Zooshikella, Hahella and Reinekea (the fatty acid profiles of which were obtained from biomass grown on MA). On the basis of the above results, strain IMCC1097T cannot be characterized as a member of any of the known genera within the order Oceanospirillales.

It is evident from the low levels of 16S rRNA gene sequence similarity (<90 %), the unique branching patterns in the phylogenetic analyses and the phenotypic characteristics
Table 1. Differential characteristics of strain IMCC1097T and other marine bacteria related to the order Oceanospirillales

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape*</td>
<td>SR</td>
<td>CR</td>
<td>LR</td>
<td>R</td>
<td>TR</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth at/with:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 °C</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>40 °C</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>8 % NaCl</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Catalase activity</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Flagella</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>Poly-β-hydroxybutyrate</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>ND or V</td>
<td></td>
</tr>
<tr>
<td>accumulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anoxic growth</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>V</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Acid from glucose</td>
<td>–</td>
<td>+</td>
<td>+</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>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>57.8</td>
<td>40–42</td>
<td>44–53</td>
<td>51.1</td>
<td>41</td>
<td>57.8</td>
<td>53–66</td>
</tr>
</tbody>
</table>

*CR, Curved rod; LR, long rod; R, rod; S, spirillum; SR, short rod; TR, thick rod.
†H. chejuensis is positive and H. ganghwensis is negative.
§Data for A. venustensis.
§§Data not available for A. jadensis.
||Data for A. borkumensis.

that the coastal marine, oligotrophic isolate cannot be assigned to any previously recognized bacterial family or genus and therefore should be characterized as a novel species within a novel genus, *Litoricola lipolytica* gen. nov., sp. nov., belonging to a novel family, *Litoricolahae* fam. nov.

**Description of Litoricola gen. nov.**

*Litoricola* (Li.to.ri’co.la. L. n. *litus* -oris seashore; L. suff. -cola from L. masc. or fem. *n. incola* inhabitant; N.L. fem. n. *Litoricola* inhabitant of the seashore).

Cells are short rods. Gram-negative. Oxidase-positive and catalase-negative. Chemoheterotrophic and facultatively aerobic. Non-motile. Nitrate is not reduced. Acid is not produced from glucose fermentation. NaCl is required for growth. The predominant fatty acids are C16:1ω7c and/or iso-C15:0 2-OH, C18:1ω7c and C10:0 3-OH. The DNA G+C content of the type strain of the type species is 57.9 mol%. Phylogenetically, the genus belongs to the family *Litoricolahae* within the order *Oceanospirillales*. The type species of the genus is *Litoricola lipolytica*.

**Description of Litoricola lipolytica sp. nov.**

*Litoricola lipolytica* [li.po.ly’ti.ca. Gr. n. *lipos* fat; Gr. adj. *lytikos* dissolving; N.L. fem. adj. *lipolytica* fat-dissolving, pertaining to esterase lipase (C8) activity of the species].

In addition to having the properties given in the genus description, the species is characterized as follows. Cells are 0.5–0.7 μm wide and 0.8–1.3 μm long, dividing by binary fission (see Supplementary Fig. S1, available in IJSEM Online). Colonies on MA are circular, smooth, convex, opaque, cream-coloured and 0.05 mm in diameter, after 1 week of incubation. Colonies are approximately 1 mm in diameter after 3 weeks incubation. Growth occurs at 15–30 °C (optimum, 25 °C), pH 5–10 (optimum, pH 7.0) and 1.5–10.0 % NaCl (optimum, 3.0–3.5 % NaCl). No growth is observed at 10 or 35 °C, at pH 4 or 10 or at 1.0 or 15 % NaCl. Aesculin is hydrolysed. β-Galactosidase activity is present. Negative for indole production, arginine dihydrolase, gelatinase and urease. Only esterase lipase (C8) activity is detected in API ZYM tests; alkaline phosphatase, esterase (C4), acid phosphatase, naphthol-AS-BI-phosphohydrolase, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin,
Table 2. Cellular fatty acid compositions (%) of strain IMCC1097T and members of related genera in the order Oceanospirillales

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>–</td>
<td>–</td>
<td>1.3–2.4</td>
<td>–</td>
<td>–</td>
<td>8.9/–/5.1/5.2</td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>–</td>
<td>6.0</td>
<td>2.4–2.8</td>
<td>2.0</td>
<td>5.5</td>
<td>–</td>
<td>–/1.1/1.4/1.7</td>
</tr>
<tr>
<td>C16:0</td>
<td>6.5</td>
<td>31.9</td>
<td>12.5–18.1</td>
<td>31.6</td>
<td>38.6</td>
<td>18.9</td>
<td>32.1/31.5/20.2/23.4</td>
</tr>
<tr>
<td>C17:0</td>
<td>–</td>
<td>–</td>
<td>1.1–2.8</td>
<td>7.7</td>
<td>–</td>
<td>–/–/2.0/–/–</td>
<td></td>
</tr>
<tr>
<td>C18:0</td>
<td>–</td>
<td>–</td>
<td>3.3–11.9</td>
<td>26.7</td>
<td>16.6</td>
<td>21.8</td>
<td>11.3/17.9/15.4/13.5</td>
</tr>
<tr>
<td>C18:1ω9c</td>
<td>20.6</td>
<td>14.5</td>
<td>&lt;1–9.4</td>
<td>19.0</td>
<td>–</td>
<td>51.2</td>
<td>22.4/47.1/19.9/20.7</td>
</tr>
<tr>
<td>C18:1ω9c</td>
<td>–</td>
<td>–</td>
<td>19.8–39.0</td>
<td>–</td>
<td>1.4</td>
<td>–</td>
<td>–/–/1.2/–</td>
</tr>
<tr>
<td>C18:1ω8c</td>
<td>–</td>
<td>–</td>
<td>9.0–10.7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–/–/–/–</td>
</tr>
<tr>
<td>C19:0</td>
<td>14.1</td>
<td>2.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>14.3/–/10.1/–</td>
<td></td>
</tr>
<tr>
<td>C12:0 3-ОH</td>
<td>–</td>
<td>5.1</td>
<td>2.5–3.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.29/–/10.7/4.9</td>
</tr>
<tr>
<td>C12:0 1-ОH</td>
<td>5.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–/–/–/–</td>
</tr>
<tr>
<td>C16:0 N alcohol</td>
<td>–</td>
<td>–</td>
<td>6.5–7.2</td>
<td>1.2</td>
<td>–</td>
<td>–</td>
<td>–/–/–/–</td>
</tr>
<tr>
<td>C17:0 10-methyl</td>
<td>–</td>
<td>–</td>
<td>0–14.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–/–/–/–</td>
</tr>
</tbody>
</table>

* C16:1ω7c or C16:1ω7c-containing fatty acid mixtures.
† C16:1ω7c and/or iso-C15:0 2-ОH.
‡ C16:1ω7c and/or iso-C16:0 2-ОH.
§ C16:1ω7c and/or C16:0 9t.
|| C18:1ω7c or C18:1ω7c-containing fatty acid mixtures.
¶ One or more of C18:1ω7c, C19:0 3-ОH, C12:0 3-ОH and C18:0 9t.

x-galactosidase, β-galactosidase, β-glucuronidase, α-gluco-
sidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mann-
osidase and α-fucosidase activities are absent. In tests for
the utilization of sole carbon sources, positive results are
obtained for the following carbon substrates: glycerol, DL-
glyceraldehyde, D-ribose, L-arabinose, L-rhamnose, D-cello-
biose, sucrose, trehalose, D-raffinose, adonitol, myo-inosi-
tol, D-xylitol, citric acid, D-glucuronic acid, pyruvic acid, L-
alanine, L-histidine, L-lysine, L-ornithine and L-serine. The
following carbon sources are utilized weakly: methylamine,
ethanol, D-galactose, D-mannose, melibiose, D-melezitose,
D-mannitol, gluconic acid, itaconic acid, propionic acid and
dL-proline. Methanol, D-xylose, D-fructose, N-acetyl-
D-glucosamine, D-glucosamine hydrochloride, D-glucose,
α-D-lactose, maltose, L-arabinol, D-sorbitol, malonic acid,
succinic acid, L-arginine, L-glutamic acid, L-glutine and L-
leucine are not utilized as sole carbon sources. Susceptible
to chloramphenicol, erythromycin, gentamicin, kanamy-
cin, rifampicin, streptomycin, tetracycline and vancomy-
cin, but resistant to ampicillin and penicillin G. The
cellular fatty acids are composed of C16:1ω7c and/or
iso-C15:0 2-ОH (42.8%), C18:1ω7c (20.6%), C10:0 3-ОH
(14.1%), an unknown fatty acid (equivalent chain-length
11.799) (6.8%), C16:0 (6.5%), C12:0 3-ОH (5.6%), C10:0
(0.9%), C14:0 (0.7%), C19:0 9т (0.8%), C18:1ω7c 11-
methy1 (0.6%), C16:1ω5c (0.4%), C12:0 (0.1%) and C12:0
3-ОH (0.1%).

The type strain, IMCC1097T (=KCCM 42360T =NBRC
102074T), was isolated from surface seawater off the coast
at Goseong, East Sea, Korea.

Description of Litoricolaceae fam. nov.

Litoricolaceae (Li.to.si.co.ia.’ce.ae. N.L. fem. n. Litoricola
type genus of the family; -aceae ending to denote a family;
N.L. fem. pl. n. Litoricolae the family of the genus
Litoricola).

The family Litoricolaceae is within the order Oceanospirillales and encompasses Gram-negative bacteria
retrieved from marine environments. Currently, the family
comprises the genus Litoricola and several uncultured

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marine bacteria. The delineation of the family is primarily determined from the phylogenetic position of the 16S rRNA gene sequence. The detailed description is the same as that given for the genus Litoricola. The type genus of the family is Litoricola.

Acknowledgements

This work was supported by a grant (KRF-2005-0150-c00528) from the Korea Research Foundation funded by the Korean Government (MOEHRD).

References


