Sneathiella chinensis gen. nov., sp. nov., a novel marine alphaproteobacterium isolated from coastal sediment in Qingdao, China

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The taxonomic position of strain LMG 23452T, which was isolated from coastal sediment from an aquaculture site near Qingdao, China, in 2000, was determined. Strain LMG 23452T comprised Gram-negative, non-spore-forming, motile rods and was found to be a halotolerant, aerobic, chemoheterotroph that produces catalase and oxidase. Comparative 16S rRNA gene sequence analysis revealed that strain LMG 23452T shared approximately 89 % sequence similarity with members of the genera Devosia, Hyphomonas, Ensifer and Chelatococcus, which belong to two different orders within the Alphaproteobacteria. Further phylogenetic analysis of the 16S rRNA gene sequence showed that strain LMG 23452T formed a separate branch within the order Rhizobiales, falling between the genera Devosia and Ensifer of the families Hyphomicrobiaceae and Rhizobiaceae, respectively. Strain LMG 23452T could be differentiated from its closest phylogenetic neighbours on the basis of several phenotypic features, including hydrolysis of the substrates starch and casein and assimilation of the carbohydrates D-glucose, D-mannose, mannitol, maltose and L-arabinose, and chemotaxonomically by the presence of the fatty acids C14:0 3-OH, C16:1ω11c, C18:1ω7c, C12:0 3-OH, C16:1ω5c and C18:1ω5c. The major fatty acids detected in strain LMG 23452T were C18:1ω7c, C18:0, C19:0 cyclo ω8c, C16:1ω7c and C17:1ω6c and the G + C content of the genomic DNA was 57.1 mol%. Therefore, the polyphasic data support the placement of strain LMG 23452T within a novel genus and species, for which the name Sneathiella chinensis gen. nov., sp. nov. is proposed. The type strain is LMG 23452T (= CBMAI 737T).

The class Alphaproteobacteria (Garrity et al., 2005a) comprises a large group of Gram-negative bacteria within the phylum Proteobacteria and is currently divided into seven orders: Caulobacterales (Henrici & Johnson, 1935), Rhodobacterales (Garrity et al., 2005b), Rhodospirillales (Pfenng & Trüper, 1971), Rickettsiales (Giesczyczkiewicz, 1939), Sphingomonadales (Yabuuchi & Kosako, 2005), Kordiimonadales (Kwon et al., 2005) and Rhizobiales (Kuykendall, 2005).

Members of the order Rhizobiales (Kuykendall, 2005) are morphologically and physiologically diverse and constitute the largest group within the α-2 subgroup of the Proteobacteria (Woese et al., 1984; Cho & Giovannoni, 2003). On the basis of 16S rRNA gene sequence analysis, the order currently comprises 11 families with validly published names: Rhizobiaceae, Bartonellaceae, Brucellaceae, Phyllobacteriaceae, Methylocystaceae, Beijerinckiaceae, Bradyrhizobiaceae, Hyphomicrobiaceae, Methyllobacteriaceae, Rhodobacteriaceae and Xanthobacteriaceae (Bowman, 2006; Garrity et al., 2006a, b, c, d; Mergaert & Swings, 2006; http://www.bacterio.cict.fr). Although these families have...
been revised and expanded by the inclusion of several new genera in recent years (Garrity et al., 2004; Lee et al., 2005), only a small number of these taxa have been described as coming from marine sources (Satomi et al., 2002; Cho & Giovannoni, 2003; Denner et al., 2003; Labbé et al., 2004; Peix et al., 2005). In the present study, we describe a novel taxon within the order Rhizobiales, which was isolated from coastal sediment from an aquaculture site.

In October 2000, strain LMG 23452\(^T\) was isolated from a sediment sample from an aquaculture site near Qingdao and was cultivated on marine agar (MA; Difco) at 28 °C. Cultures were maintained on MA slants at room temperature and stock cultures were kept in tryptone soy broth (Oxoid) supplemented with 1 % (w/v) NaCl (TNB) and 20 % (v/v) glycerol and stored at −70 °C. Colony morphology was recorded on MA after 48 h incubation at 28 °C. Cellular morphology was determined by phase-contrast microscopy (Axiopt; Zeiss) at ×1000 magnification. Gram staining was performed using the modified method of Hucker & Conn (1923). Stained cells were observed using a light microscope (Microlux-11; Kyowa) at a magnification of ×1000. Cells of strain LMG 23452\(^T\) were found to be Gram-negative rods.

For phenotypic tests, the strain was grown on MA for 48 h at 28 °C and cells were resuspended in saline for use as an inoculum. Tolerance of 3, 5, 7 and 10 % (w/v) NaCl was assessed on appropriately modified tryptone soy agar (Oxoid). Growth in the absence of NaCl was assessed on plate count agar (PCA; Oxoid). Inoculated plates were incubated at 28 °C for up to 5 days. The effects of different temperatures on growth were assessed on tryptone soy agar plates supplemented with 1.0 % (w/v) NaCl (TNB) and incubated at 4, 28, 30, 37, 45 and 50 °C. Anaerobiosis was determined on MA in an anaerobic chamber (Merck) containing the anaerobic catalyst Anaerocult (Merck) prepared according to the manufacturer’s instructions. The chamber was incubated at 28 °C and examined after 7 days. Motility was assessed in a semi-solid medium prepared according to MacFaddin (1976). The tube was incubated at 25 °C for 5 days. The reduction of nitrate was assessed in nitrate broth, prepared according to the method of Cowan & Steel (1974), and incubated at room temperature for 10 days. Oxidase and catalase activities were determined by using standard methods. Tests for the hydrolysis of casein, starch, tyrosine, aesculin and Tweens 20, 40, 60 and 80 were performed on TNA plates; the substrate concentrations and incubation conditions were as described by Cowan & Steel (1974). Insoluble-dye-linked polysaccharides (galactan, arabinan, xylan, cellulose or pullulan; Megazyme International) were added to a base medium of MA at a concentration of 0.05 % (w/v) and autoclaved at 115 °C for 15 min. The plates were incubated at 25 °C for 3 days. API 20NE and API ZYM test kits (bioMérieux) were inoculated with strain LMG 23452\(^T\), using the appropriate suspension medium, and incubated according to the manufacturer’s instructions.

Antibiotic sensitivity was assessed as follows: a cell suspension (≈ 10\(^7\) cells ml\(^{-1}\)) was swabbed over the surface of Iso-Sensitest agar (Oxoid) plates supplemented with 1 % (w/v) NaCl to create a uniform lawn before aseptic placement of antibiotic discs (M5 and M27, Mastring; Mast Laboratories) onto the agar surface. The inoculated plates were incubated overnight at 28 °C.

Fatty acid methyl esters of strain LMG 23452\(^T\) were obtained from 40 mg cells. Saponification, methylation and extraction were performed according to the procedures of Miller (1982) and Kuykendall et al. (1988). Separation and analysis were performed essentially as described by Rivas et al. (2003), but with a slight modification to the gas chromatographic parameters (injection-port temperature, 240 °C; detector temperature, 300 °C).

The DNA G+C content (mol%) was determined by HPLC (Tamaoka & Komagata, 1984). The almost-complete 16S rRNA gene sequence (1412 nt) of strain LMG 23452\(^T\) was obtained using the universal primers 27f and 1492r (MWG Biotech; Lane, 1991). The PCR amplification mixture (50 μl) comprised 2.5 μl (5 pmol μl\(^{-1}\)) each of primers 27f and 1492r, 2.5 μl MgCl\(_2\) (50 mM; Bioline), 10 μl Taq mix [i.e. 5 μl (10 mM NH\(_4\)Cl\(_2\)); 10 μl each of dATP, dTTP, dGTP, dCTP (100 mM); 4.6 μl sterile Millipore H\(_2\)O], 31.2 μl sterile Millipore water and 1.0 μl DNA template. PCR amplification was carried out on a Bio-Rad iCycler (version 3.021). Template DNA was initially denatured at 95 °C for 5 min and the machine paused for the addition of 0.3 μl Taq enzyme (BIOTAQ; Bioline). The PCR was resumed for a further 35 cycles of 95 °C for 1 min, 56 °C for 1 min and 72 °C for 2 min, with a final extension at 72 °C for 10 min. The PCR product was subjected to polyethylene glycol precipitation (Embley, 1991). Purified PCR products (2 μl) were mixed with 1 μl ABI Prism Big Dye Terminator cycle sequencing ready reaction mix V1.1 with AmpliTaq DNA polymerase (Applied Biosystems), 1 μl 5× ABI sequencing buffer (Sigma), 2.4 μl sterile filtered water and 3.2 μl (1 pmol μl\(^{-1}\)) of one of the following universal primers designed by Lane (1991), i.e. 27f (5′-AGAGTTT-GATCMTGGCTCAG-3′), 519r, 342f, 522f, 926f, 907r, 1141f, 1100r, 1492r (5′-TACCGYTACCTTGTTACGACT-3′) (MWG Biotech). Reactions were carried out in a thermocycler. The program consisted of a denaturation step at 98 °C for 5 min, followed by 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. The sequencing products were purified with an ethanol/EDTA/sodium acetate precipitation solution (53 μl 96 % ethanol: 2 μl EDTA: 2 μl sodium acetate) in 96-well reaction plates (Applied Biosystems) and resuspended in 25 μl template suppression reagent (Applied Biosystems), heated at 95 °C for 2 min and then analysed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The resulting forward and reverse sequences were analysed and aligned using the STaden package (Staden, 1996). A BLASTN (version 2.2.12) search was carried out to compare the corrected 16S rRNA gene sequence with those held in the National Library of
Medicine databases (Bethesda, MD, USA; Altschul et al., 1997). The sequences were aligned, manually cropped using CLUSTAL W (Thompson et al., 1994) and a phylogenetic tree was constructed. Sequences were uploaded into CLUSTAL_X (version 1.81; Jeanmougin et al., 1998) in FASTA format for multiple alignment. A tree was calculated using the neighbour-joining method (Saitou & Nei, 1987), with bootstrapping determined for 1000 replicates. The tree was visualized using TREEVIEW (Page, 1996) and rooted using a suitable outgroup.

A BLASTN search using the almost-complete 16S rRNA gene sequence of strain LMG 23452T (1412 bp) placed it among members of the class Alphaproteobacteria. The closest phylogenetic neighbours were Chelatococcus asaccharovorans DSM 6462T, Devosia riboflavina DSM 7230T and Hyphomonas polymorpha DSM 2665T, which showed 89 % 16S rRNA gene sequence similarity with the novel strain. A neighbour-joining tree (Fig. 1) revealed that strain LMG 23452T grouped most closely with an uncultured bacterial clone, D101, derived from a deep-sea sediment sample of a western Pacific warm pool in China (GenBank accession number AY375134). The closest cultured relatives of strain LMG 23452T belonged to two genera, Devosia (Nakagawa et al., 1996) and Ensifer (Casida, 1982), of the families Hyphomicrobiaceae (Babudieri, 1950) and Rhizobiaceae (Conn, 1938), respectively. Strain LMG 23452T formed a separate branch within the order Rhizobiales, showing less than 90 % 16S rRNA gene sequence similarity with respect to its neighbours (with high levels of bootstrap support). Clearly this rather low level of similarity suggests that strain LMG 23452T belongs to a novel taxonomic group.

Interestingly, the genus Chelatococcus (Auling et al., 1993) was placed within the order Rhodobacterales (Fig. 1), which conflicts with the currently accepted taxonomic outline of the Alphaproteobacteria (Garrity et al., 2005a). However, a recent phylogenetic analysis of the Alphaproteobacteria by Lee et al. (2005) placed Chelatococcus at an intermediate position within the order Rhizobiales, making it difficult to define its taxonomic hierarchy. There are, however, numerous 16S rRNA gene sequence signatures that distinguish the genus Sneathiella from the closely related genera Devosia and Ensifer. Although there are differences in the base sequences of all three genera, e.g. at positions 155, 166, 441, 681 and 709, at various other nucleotide positions the base sequences of Devosia and Ensifer are identical, whereas those pertaining to strain LMG 23452T are different. Moreover, at nucleotide positions 1000–1003, (Escherichia coli numbering) strain LMG 23452T contains the signature sequence GTAG, followed by an insertion of the bases TT at position 1003 and 1004, to read GTAGTTT. This sequence (GTAGTTT) is different from the signature sequences of the genera Devosia and Ensifer (results not shown). At nucleotide positions 1262–1265 and 1270–1273, strain LMG 23452T contains the signature sequences AGGG and CCCT, respectively, which are also different from those in members of the genera Devosia and Ensifer. In addition, there is a base deletion at position 1453–1454 (Escherichia coli numbering) in strain LMG 23452T that is not present in members of the genera Devosia or Ensifer. Clearly there are numerous signature sequences that distinguish strain LMG 23452T from its closest phylogenetic relatives.

Fig. 1. Neighbour-joining tree, based on almost-complete 16S rRNA gene sequences (~1312 bp) of strain LMG 23452T and related reference strains. Kordiimonas gwan- gyangensis GW14-5T was used as the root. Numbers on branches refer to confidence limits (expressed as percentages) estimated from a bootstrap analysis based on 1000 replicates. Accession numbers are shown in parentheses. Bar, 0.01 substitutions per nucleotide.
The complete biochemical and antibiogram data for strain LMG 23452\textsuperscript{T} are given in the species description. The phenotypic features that differentiate strain LMG 23452\textsuperscript{T} from its closest phylogenetic relatives are provided in Table 1. Under comparable test conditions, strain LMG 23452\textsuperscript{T} can be distinguished from the type species of the genus *Devosia* (*i.e.* *D. riboflavina*) and from members of the genus *Ensifer* by its inability to utilize N-acetylglucosamine or to assimilate the carbohydrates D-glucose, L-arabinose, D-mannose, mannitol and maltose. Unlike strain LMG 23452\textsuperscript{T}, members of the genus *Ensifer*, including the type species of the genus, *Ensifer adhaerens*, are able to utilize malate, but are unable to hydrolyse casein (Young, 2003). Similarly, gentamicin resistance (10 \textmu g), kanamycin resistance (30 \textmu g) and \(\beta\)-galactosidase production are observed in strains of *Ensifer adhaerens* (Wang et al., 2002; Willems et al., 2003) and members of the genus *Devosia* (Vanparys et al., 2005), but not in strain LMG 23452\textsuperscript{T}. Taxa belonging to the genus *Devosia* are able to produce the enzymes \(N\)-acetyl \(\beta\)-glucosaminidase and cystine arylamidase and utilize the substrate caprylate (Vanparys et al., 2005), whereas these traits are not observed in strain LMG 23452\textsuperscript{T}. Moreover, the type species of the genus *Devosia*, *D. riboflavina* (LMG 2277\textsuperscript{T}), is able to produce the enzymes \(\alpha\)-fucosidase, \(\alpha\)-mannosidase and \(\alpha\)-galactosidase, unlike strain LMG 23452\textsuperscript{T}. Clearly, there are several phenotypic features of the novel strain that distinguish it from its closest phylogenetic relatives.

The DNA G+C content of strain LMG 23452\textsuperscript{T} was 57.1 mol\%. In the emended description of the genus *Ensifer* (Young, 2003), the DNA G+C content is 57–66 mol\% and that of members of the genus *Devosia* falls within the range 61–63 mol\% (Rivas et al., 2003); clearly, the DNA G+C content of strain LMG 23452\textsuperscript{T} differs markedly from those of its closest phylogenetic relatives (Table 1).

The use of the fatty acid profile and the MIDI database could not provide an accurate identification of LMG 23452\textsuperscript{T}, which reinforced the notion that this strain belonged to a novel taxonomic group. A comparison of the fatty acid profile of strain LMG 23452\textsuperscript{T} with the profiles for its closest phylogenetic neighbours, obtained under comparable test conditions (Table 2), revealed that all strains contained C\textsubscript{16:0} with the majority of strains containing C\textsubscript{18:1}\textit{\textcircled{v}}7c and C\textsubscript{19:0} cyclo \textit{\textcircled{c}}8c. According to Martínez-Checa et al. (2005), the presence of cis-11 octadecenoic acid (i.e. C\textsubscript{18:1}\textit{\textcircled{v}}7c) as the principal fatty acid is characteristic of taxa within the *Alphaproteobacteria*, whilst the presence of C\textsubscript{19:0} cyclo \textit{\textcircled{c}}8c

\begin{table}[h]
\centering
\caption{Some phenotypic features useful for differentiating strain LMG 23452\textsuperscript{T} from its closest phylogenetic neighbours}
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Characteristic} & \textbf{1} & \textbf{2} & \textbf{3} & \textbf{4} & \textbf{5} & \textbf{6} & \textbf{7} & \textbf{8} & \textbf{9} \\
\hline
\text{N-Acetylglucosamine} & $-$ & $+$ & $+$ & $+$ & $+$ & $+$ & $-$ & $+$ & $-$ * \\
\text{Starch hydrolysis} & $+$ & $+$ & $+$ & $+$ & $+$ & $+$ & $+$ & $+$ & $+$ \\
\text{Assimilation of:} & & & & & & & & & \\
\text{D-Glucose} & $-$ & $+$ & $+$ & $+$ & $+$ & $+$ & $-$ & $+$ & $+$ \\
\text{L-Arabinose} & $-$ & $+$ & $+$ & $+$ & $+$ & $+$ & $-$ & $+$ & $+$ \\
\text{D-Mannose} & $-$ & $+$ & $+$ & $+$ & $+$ & $+$ & $-$ & $+$ & $+$ \\
\text{Mannitol} & $-$ & $+$ & $+$ & $+$ & $+$ & $+$ & $-$ & $+$ & $+$ \\
\text{Maltose} & $-$ & $+$ & $+$ & $+$ & $+$ & $+$ & $-$ & $+$ & $+$ \\
\text{Malate} & $-$ & $+$ & $+$ & $+$ & $+$ & $+$ & $-$ & $+$ & $+$ \\
\text{Caprylate} & $-$ & $-$ & $-$ & $-$ & $-$ & $-$ & $-$ & $-$ & $+$ \\
\text{DNA G+C content (mol\%)} & 57.1 & 59.9–63.8§ & 62–63§ & 62–63§ & 60.8–61.6§ & 61.7–62.3ll & 61.9* & 61.4¶ & 62.4§ \\
\hline
\end{tabular}
\footnotesize
*Data from Vanparys et al. (2005).
†Data from Young (2003).
§Data from Chen et al. (1988).
¶Data from de Lajudie et al. (1994).
llData from Willems et al. (2003).
Data from Nakagawa et al. (1996).
#Data from Rivas et al. (2003).
\end{table}
Table 2. Total fatty acid content (%) of strain LMG 23452T and related species

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<td>3.44</td>
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<td>—</td>
<td>4.59</td>
<td>4.79</td>
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<tr>
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<td>—</td>
<td>&lt;1.00</td>
<td>&lt;1.00</td>
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<tr>
<td>C20:1ω9t</td>
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<td>—</td>
<td>0.12</td>
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<td>C20:1ω6,9,12c</td>
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<td>—</td>
<td>3.28</td>
<td>2.00</td>
<td>—</td>
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</tr>
<tr>
<td>Summed feature 1</td>
<td>—</td>
<td>1.54</td>
<td>2.96</td>
<td>2.27</td>
<td>0.63</td>
<td>0.42</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Summed feature 2</td>
<td>1.66</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7.62</td>
<td>7.70</td>
<td>—</td>
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<tr>
<td>Summed feature 3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>71.45</td>
<td>79.40</td>
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<tr>
<td>Summed feature 5</td>
<td>—</td>
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<td>—</td>
<td>—</td>
<td>0.08</td>
<td>—</td>
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</tbody>
</table>

in significant amounts is thought to be typical of members of the order Rhizobiales (Rivas et al., 2005). However, a 16S rRNA gene sequence comparison of strain LMG 23452T in BLASTN revealed 89% similarity with members of the genus Hyphomonas (order Rhodobacterales) (results not shown). Table 2 shows the absence of the fatty acid C19:0 cyclo ω8c in members of the genus Hyphomonas. In spite of the fact that strain LMG 23452T contained significant amounts of C19:0 cyclo ω8c, the fatty acid profile was distinct from those obtained for other members of the Rhizobiales, such as the genera Devosia and Ensifer (Table 2). The fatty acids C20:1ω6,9,12c and summed feature 3 (C18:1ω7cω9t/ω12t, C18:1ω7cω9tω12t) (Table 2) are common to all species of Ensifer (Tighe et al., 2000), but were not detected in the novel strain, which confirms that LMG 23452T is not a member of this genus. Similarly, members of the genus Devosia contained the fatty acids C10:0 3-OH and C18:0 which were not detected in strain LMG 23452T.
Clearly, the fatty acids C\textsubscript{14:0} 3-OH, C\textsubscript{16:1}ω9t11c, C\textsubscript{16:0}ω5c and C\textsubscript{18:1}ω5c, were present only in strain LMG 23452\textsuperscript{T}.

On the basis of the polyphasic taxonomic data obtained in this study, we conclude that strain LMG 23452\textsuperscript{T} is a representative of a hitherto unknown marine taxon of the order Rhizobiales, class Alphaproteobacteria, for which the name *Sneathiella chinensis* gen. nov., sp. nov., is proposed.

**Description of Sneathiella gen. nov.**

*Sneathiella* (Sneath.i’la. N.L. fem. dim. n. *Sneathiella* honouring the British microbiologist Peter H. A. Sneath for his contributions to bacterial taxonomy).

Gram-negative, motile, aerobic, small, non-spore-forming and rod-shaped. Cells are oxidase- and catalase-positive. Cells grow at temperatures in the range 4–37 °C. NaCl is not required for growth. Colonies are beige, low convex, glossy, smooth, irregular and 0.5–2.0 mm in diameter on MA. Major cellular fatty acids are C\textsubscript{18:1}ω7c (46.2 %), C\textsubscript{16:0} (17.2 %), C\textsubscript{19:0} cyclo ω8c (9.8 %), C\textsubscript{16:1}ω7c (6.9 %) and C\textsubscript{17:1}ω6c (5.6 %). 16S rRNA gene sequence analysis indicates that it is phylogenetically related to members of the γ-subgroup of the Proteobacteria. The type species is *Sneathiella chinensis*.

**Description of Sneathiella chinensis sp. nov.**

*Sneathiella chinensis* (chi.nen’sis. N.L. fem. adj. *chinensis* pertaining to China, where the type strain was isolated).

In addition to the properties described for the genus, the following properties apply. After 48 h on MA, colonies (diameter 0.5–2.0 mm) are beige, low convex, glossy, smooth, irregular and butyrous. Cells are Gram-negative and motile. No growth occurs at 45 °C. NaCl is not required for growth. Colonies are beige, low convex, glossy, smooth, irregular and 0.5–2.0 mm in diameter on MA. 

Other properties are listed in Table 2. The DNA G+C content of the type strain is 57.1 mol%.

The type strain, LMG 23452\textsuperscript{T} (= CBMAI 737\textsuperscript{T}), was isolated from sediment from a coastal aquaculture site at Xianlangzhui, Qingdao, China.

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**References**


