The genus Abiotrophia (Kawamura et al.) is not monophyletic: proposal of Granulicatella gen. nov., Granulicatella adiacens comb. nov., Granulicatella elegans comb. nov. and Granulicatella balaenopteræa comb. nov.

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The genus Abiotrophia currently includes four species, Abiotrophia defectiva, Abiotrophia adiacens, Abiotrophia balaenopteræa and Abiotrophia elegans. Recent 16S rRNA gene sequencing studies have demonstrated that the genus is not monophyletic and is in need of taxonomic revision. Phylogenetically, the genus Abiotrophia consists of two distinct lines, A. defectiva, the type species of the genus, and a robust group consisting of A. adiacens, A. balaenopteræa and A. elegans. Therefore, it is formally proposed that the genus Abiotrophia should be restricted to A. defectiva and that A. adiacens, A. balaenopteræa and A. elegans should be reclassified in a new genus, Granulicatella, as Granulicatella adiacens comb. nov., Granulicatella balaenopteræa comb. nov. and Granulicatella elegans comb. nov.

Keywords: Abiotrophia, Granulicatella gen. nov., taxonomy, phylogeny, nutritionally variant streptococci

Nutritionally variant streptococci (NVS) were originally described by Frenkel & Hirsch (1961) as a new type of viridans group streptococcus that exhibited satellitism around the colonies of other bacteria. Throughout the literature, these organisms have been referred to by a variety of terms, such as NVS (Cooksey et al., 1979; Bouvet et al., 1981), nutritionally deficient streptococci (Bouvet et al., 1980), satelliting streptococci (McCarthy & Bottone, 1974), vitamin B$_{12}$-dependent streptococci (Carey et al., 1975) and pyridoxal-dependent streptococci (Roberts et al., 1979), because of their fastidious nutritional requirements. NVS are members of the normal flora of the human pharynx and the human urogenital and intestinal tracts (Ruoff, 1991). Like other viridans group streptococci, NVS cause sepsis and bacteraemia and are responsible for a substantial proportion of cases of infectious endocarditis (Ruoff, 1991; Bouvet, 1995). The taxonomic status of these fastidious organisms was greatly clarified by Bouvet et al. (1989), who demonstrated the existence of two distinct species within the NVS by chromosomal DNA–DNA hybridizations, which were named Streptococcus adjacens and Streptococcus defectivus. The hybridization studies of Bouvet et al. (1989) revealed that S. adjacens and S. defectivus shared less than 10% DNA homology with each other and with other streptococcal species. Kawamura et al. (1995), using 16S rRNA gene sequencing, showed that S. adjacens and S. defectivus formed a distinct clade that was phylogenetically far-removed from the streptococci and proposed that these species be transferred to a new genus, Abiotrophia, as Abiotrophia adiacens and Abiotrophia defectiva. The phylogenetic separateness of these NVS from authentic streptococci, together with nutritional considerations, satellitism and pyrrolidonyl arylamidase production, were the primary reasons for the creation of the genus Abiotrophia.

It is pertinent to note that, in the 16S rRNA sequence analysis conducted by Kawamura et al. (1995), the exclusion of NVS organisms from the genus Streptococcus was justified on phylogenetic grounds (e.g. from tree topology considerations and 16S rRNA sequence divergence values of generally more than 11% from streptococcal species). Although it was evident from this study (Kawamura et al., 1995) that NVS organisms shared higher 16S rRNA relatedness with certain other catalase-negative taxa (e.g. Aero-
Abiotrophia, the closest relatives. The tree was constructed by using the neighbour-joining method and is based on a comparison of approx. 1320 nucleotides. Bootstrap values, expressed as a percentage of 200 replicates, are given at the branching points. Bar, 1%.

Table 1. Characteristics that differentiate Granulicatella species from Abiotrophia defectiva

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A. defectiva</th>
<th>G. balaenopterae</th>
<th>G. adiacens</th>
<th>G. elegans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production of acid from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pullulan</td>
<td>v</td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>Sucrose</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>Tagatose</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>Trehalose</td>
<td></td>
<td>+</td>
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<td>Hydrolysis of:</td>
<td></td>
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<td></td>
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<tr>
<td>Hippurate</td>
<td></td>
<td></td>
<td></td>
<td>v</td>
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<tr>
<td>Production of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine dihydrolase</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td></td>
<td>+</td>
<td></td>
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<tr>
<td>β-Glucuronidase</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Murein type</td>
<td>A1α</td>
<td>A4β</td>
<td>A3α</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, Not determined, v, variable.

coccus, Carnobacterium, Enterococcus) than with streptococci, aside from a 7% 16S rRNA sequence divergence between A. adiacens and A. defectiva, there was little evidence to suggest that the newly delineated genus was not monophyletic. Since the description of Abiotrophia by Kawamura et al. (1995), two further species of this genus have, however, been characterized, Abiotrophia elegans (Roggenkamp et al., 1998), from a patient with endocarditis, and Abiotrophia balaenopterae (Lawson et al., 1999), isolated from a minke whale (Balaenoptera acutorostrata). Interestingly, both new species display a closer phylogenetic affinity with A. adiacens (16S rRNA sequence divergence approximately 3%) than with A. defectiva (sequence divergence approximately 7%), and it is evident that there are two distinct lines within the
genus Abiotrophia, namely A. defectiva and a group consisting of A. adiacens, A. balaenopterae and A. elegans. Furthermore, it is also very apparent that A. defectiva, the type species of the genus, is phylogenetically closer to several non-Abiotrophia species including Globoicatella sanguinis, a chain-forming coccus described by Collins et al. (1992) but not included in the study of Kawamura et al. (1995), and species of the recently described genera Faccklamia (Collins et al., 1997, 1998), Eremococcus (Collins et al., 1999b) and Ignavigranum (Collins et al., 1999a), rather than to A. adiacens and its close relatives. A tree constructed by the neighbour-joining method summarizing current knowledge of the phylogenetic relationships of Abiotrophia species is shown in Fig. 1, and it is evident from this analysis that A. adiacens, A. balaenopterae and A. elegans form a natural and robust group that is distinct from A. defectiva. The close affinity between A. adiacens, A. balaenopterae and A. elegans and their separateness from A. defectiva has been demonstrated in several studies (Collins et al., 1998, 1999a, b; Lawson et al., 1999) and is consistently reproduced using different treeing methods including parsimony and maximum-likelihood. The genus Abiotrophia as presently constituted is thus non-monophyletic and the species A. adiacens, A. balaenopterae and A. elegans should no longer be retained within this genus. On the basis of 16S rRNA tree topology and sequence divergence considerations, we are of the opinion that A. defectiva should be restricted to the type species A. defectiva, and that A. adiacens and close relatives form a phylogenetic group worthy of separate generic status. Therefore, we formally propose that A. adiacens (Kawamura et al., 1995), A. balaenopterae (Lawson et al., 1999) and A. elegans (Roggenkamp et al., 1998) be reclassified in a new genus, Granulicatella gen. nov., as Granulicatella adiacens comb. nov., Granulicatella balaenopterae comb. nov. and Granulicatella elegans comb. nov.

Tests that are useful in distinguishing Abiotrophia and Granulicatella species are shown in Table 1.

**Description of Granulicatella adiacens comb. nov.**

This description is based essentially on that given by Bouvet et al. (1989). Cells are Gram-positive cocci. Non-motile, non-sporulating, catalase- and oxidase-negative. Facultatively anaerobic with complex growth requirements. Grows as satellite colonies adjacent to Staphylococcus epidermidis on horse-blood trypticase soy agar and on stored sheep-blood agar. α-Haemolytic on sheep-blood agar. Tiny colonies up to 0.2 mm in diameter are formed on fresh sheep-blood agar or on blood agar supplemented with pyridoxal or L-cysteine. Grows in Todd-Hewitt broth (THB) enriched with 10 mg pyridoxal l⁻¹ or 100 mg cysteine l⁻¹. Grows in CDMM semi-synthetic medium (Bouvet et al., 1981) and produces a red chromophore visualized by boiling the bacterium at pH 2 for 5 min. Cellular morphology depends upon the conditions of growth: the organism is pleomorphic with chains including cocci, coccobacilli and globular and rod-shaped cells when it is grown in cysteine- or pyridoxal-supplemented broth. Small ovoid cocci (diameter 0.4–0.6 µm) occur singly, in pairs or in chains of variable length in CDMM medium. Some tendency towards rod formation is observed in the stationary phase. Lactic acid is the predominant acid produced. Resistant to optochin and susceptible to vancomycin. No production of exopolysaccharide on 5% sucrose. Produces pyrrolidonyl arylamidase. Alkaline phosphatase, α-galactosidase, β-galactosidase, α-galactosidase, β-galactosidase and β-galactosidase are not produced. Tagatose and sucrose are fermented. D-Ribose, L-arabinose, D-mannitol, melibiose, melezitose, pullulan, sorbitol, lactose, D-raffinose, trehalose, starch and glycogen are not fermented. Inulin is fermented by some strains. Some strains produce β-glucuronidase. Leucine aminopeptidase is produced. Arginine and hippurate are not hydrolysed. Acetoin is not produced. Contains an A3 type cell wall murein. Isolated from the throat flora, urine and blood of patients with endocarditis. The G+C content of the DNA is 36.6–37.4 mol%. The type strain is ATCC 49175T (= CIP 103243T).

**Description of Granulicatella elegans comb. nov.**

This description is based essentially on that given by Roggenkamp et al. (1998). Cells stain Gram-positive. Cellular morphology is dependent on nutritional state. In sufficiently supplemented nutritional conditions. Elongated, swollen cells may be observed. Some organisms are fastidious and grow poorly in media used routinely for streptococci, e.g. blood trypticase soy agar, and require supplements such as L-cysteine and/or pyridoxal. Some grow as satellite colonies adjacent to other organisms such as Staphylococcus epidermidis. Non-motile and non-sporulating. Facultatively anaerobic and catalase- and oxidase-negative. Lactic acid is produced from glucose metabolism. Does not produce gas from glucose. Growth does not occur at 10 or 45 °C. Pyrrolidonyl arylamidase and leucine arylamidase are produced. Alkaline phosphatase, α-galactosidase, β-galactosidase and urease are not produced. Acetoin is not produced. The G+C content of DNA is approximately 36–37 mol%. The type species of the genus Granulicatella is Granulicatella adiacens.

International Journal of Systematic and Evolutionary Microbiology 50

367
broth, the bacterium appears coccoid in short chains. Lack of appropriate growth factors results in pleomorphism and the appearance of elongated, swollen forms. The organism is non-motile, non-spore-forming and catalase- and oxidase-negative and grows as a facultative anaerobe with complex growth requirements. It grows as satellite colonies adjacent to Staphylococcus epidermidis on trypticase soy blood agar plates with α-haemolysis. Tiny colonies up to 0.2 mm in diameter are formed on Schaedler sheep-blood agar plates after 48 h, but only minimal growth is visible on chocolate agar plates. Growth occurs at 27 and 37 °C but not at 20 or 42 °C. Grows in THB or casein-soy peptone bouillon supplemented with 0-01% L-cysteine hydrochloride. It does not grow in THB or casein-soy peptone bouillon supplemented with 0.001% pyridoxal hydrochloride. Produces a red chromophore visualized by boiling the micro-organism at pH 2 for 5 min. Pyrrolidonyl arylamidase, glucosidase, glycyl-tryptophane arylamidase, and catalase and oxidase-negative. The cell wall contains an α-glucosidase, β-glucuronidase, β-mannosidase, β-glucosidase, glycylic-tryptophane arylamidase, β-mannosidase and are not fermented. Raffinose and sucrose may or may not be hydrolysed. Raffinose and sucrose may or may not be fermented. Trehalose, inulin, pullulan, tagatose, lactose, starch, glycogen, D-arabitol, sorbitol, mannitol, melibiose, melezitose, L-arabinose and ribose are not fermented. Isolated from a patient with endocarditis. Habitat is not known. The type strain is DSM 11693T.

**Description of Granulicatella balaenopterae comb. nov.**

This description is based on that given by Lawson et al. (1999). Cells consist of Gram-positive cocci occurring as single cells, in pairs or in short chains. Cells are non-spore-forming and non-motile. Tiny colonies up to 0.2 mm in diameter are formed on blood agar at 37 °C. Facultatively anaerobic and catalase- and oxidase-negative. The bacterium produces acid from glucose, maltose, pullulan and trehalose. Acid is not produced from L-arabinose, D-arabitol, cyclodextrin, glycogen, lactose, mannitol, melibiose, melezitose, D-raffinose, sucrose, sorbitol, tagatose or D-xylene. Arginine dihydrolase, pyrogallaric acid arylamidase (weak action). N-acetyl-glucosaminidase, ester lipase (C5), leucine arylamidase and urease (weak action) activities are detected. Alkaline phosphatase, acid phosphatase, alanine-phenylalanine-proline arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, glycylic-tryptophane arylamidase, α-mannosidase, chymotrypsin, trypsin, α-fucosidase and pyrazinamidase activities are not detected. Aesculin is hydrolysed. Hippurate and gelatin are not hydrolysed. Nitrate is not reduced. The cell wall contains an L-Orn–D-Asp directly cross-linked murein (type A4β). The G+C content of DNA of the type strain is 37 mol%. Isolated from minke whale. Habitat is not known. The type strain is CCUG 37380T.

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**References**


