**Williamsia spongiae** sp. nov., an actinomycete isolated from the marine sponge *Amphimedon viridis*

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### Abstract

A novel actinobacterium, designated isolate B138ᵀ, was isolated from the marine sponge *Amphimedon viridis*, which was collected from Praia Guacé (São Paulo, Brazil), and its taxonomic position was established using data from a polyphasic study. The organism showed a combination of chemotaxonomic and morphological characteristics consistent with its classification in the genus *Williamsia* and it formed a distinct phyletic line in the *Williamsia* 16S rRNA gene tree. It was most closely related to *Williamsia serinedens* DSM 45037ᵀ and *Williamsia delignens* DSM 44902ᵀ (99.0 % 16S rRNA gene sequence similarity) and *Williamsia maris* DSM 44693ᵀ (97.5 % 16S rRNA gene sequence similarity), but was distinguished readily from these strains by the low DNA–DNA relatedness values (62.3–64.4 %) and by the discriminatory phenotypic properties. Based on the data obtained, the isolate B138ᵀ (≈CBMAI 1094ᵀ ≈DSM 46676ᵀ) should be classified as the type strain of a novel species of the genus *Williamsia*, for which the name *Williamsia spongiae* sp. nov. is proposed.

The genus *Williamsia* was proposed by Kämpfer et al. [1] and was classified as a group of mycolic-acid-containing actinomycetes within the suborder * Corynebacterineae* [2]. This taxon also contains the genera *Dietzia*, *Gordonia*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Skermania*, *Tsukamurella* and *Turicella*; the latter lacks mycolic acids [3, 4]. The genus *Williamsia* was placed together with the genera *Millsia* and *Skermania* between the families *Nocardiaceae* and *Gordoniaceae* [1, 5]. Based on its mycolic acid profile, short chain mycolic acids (carbon chain length C₅₀–C₆₄) are present in the genus *Williamsia* and this taxon occupies an intermediate position between *Rhodococcus* (mycolic acid chain length C₁₄–C₄₅) and *Gordonia* (mycolic acid chain length C₅₄–C₆₆) [1].

Species of this genus are Gram-positive cells with non-spore-forming, short rod elements [1]. These organisms are characterized by the presence of *meso*-diaminopimelic acid, arabinose, galactose, mannose and ribose as the major cell-wall sugars. The polar lipid profile has phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol (phospholipid type II) as major components [6]. The fatty acid profile is dominated by palmitic acid, hexadecenoic acid, oleic acid and tuberculostearic acid (fatty acid type 1b sensu) [7]. The predominant menaquinone types are MK-9(H₂), MK-8(H₂) [1, 8–10] and MK-7(H₂).

At the time of writing, the genus encompasses nine species with validly published names (www.bacterio.net/williamsia.html): *Williamsia muralis* [1], *Williamsia maris* [8], *Williamsia delignens* [9], *Williamsia marianensis* [11], *Williamsia serinedens* [10], *Williamsia faeni* [12], *Williamsia phyllosphaeae* [13], *Williamsia limnetica* [14] and *Williamsia sterculiae* [15].

Members of this genus are widely distributed in various environments such as human blood [9], soil [10], meadow hay [12], deep-sea sediments [8, 11], indoor building materials [1], leaf surfaces [13], lake sediment [14] and the surfaces of a stem of a medicinal plant [15].

The reasons for isolating actinomycetes include the importance of sponge-bacteria symbioses as a prominent field in marine biology and biotechnology, with implications ranging from a fundamental understanding of sponge ecology and the gaining of further insights into the evolution of prokaryote-eukaryote symbiotic interactions. The prospecting of natural products and synthesis of bioactive metabolites are further reasons for isolating actinomycetes. At the time of writing, there is no information available about the

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**Keywords:** Williamsia spongiae; Polyphasic taxonomy; São Paulo state; marine sponge.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA sequence of *Williamsia spongiae* B138ᵀ (=CBMAI 1094ᵀ ≈DSM 46676ᵀ) is JN615440. Four supplementary figures are available with the online Supplementary Material.
biotechnological applications of bacteria belonging to the genus *Williamsia*.

The present study was designed to establish, using a polyphasic approach, the taxonomic position of strain B138\(^\text{T}\), which was isolated from a marine sponge, *Amphimedon viridis*, collected in São Paulo, Brazil. The resultant data shows that the isolate forms a novel centre of taxonomic variation in the genus *Williamsia* for which we propose the name *Williamsia spongiae* sp. nov.

Strain B138\(^\text{T}\) was isolated from *Amphimedon viridis*, a marine sponge, which has been related to antimicrobial activity. It was collected in January 2007 in beach areas named Praia Guaéc (23 149° S, 45 125° W) in the São Sebastião region, São Paulo State, Brazil, at depths of between 5 and 10 m. Treatment of the sample and preparation of media for bacterial isolation were performed as described by Menezes et al. [16]. Isolate B138\(^\text{T}\) was recovered from Tryptic Soy Agar plates [TSA; Oxoid; prepared with 80% (v/v) artificial seawater], which had been inoculated with a serially diluted suspension of grinded *Amphimedon viridis* sponge and incubated at 25 °C for 4 weeks. The organism was maintained on TSA plates at 4 °C and as a cell suspension in glycerol (20 % w/v) at −80 °C. The biomass for chemotaxonomic studies was grown in shake flasks of ISP2 broth (International Streptomycyces Project medium 2) [17] for 7 days at 30 °C, it was harvested by centrifugation and washed twice in distilled water.

The phylogenetic position of strain B138\(^\text{T}\) was inferred by 16S rRNA gene sequence analysis. Genomic DNA was extracted according to a modified protocol of Van Soolingen et al. [18] and PCR amplification and 16S rRNA gene sequencing were achieved using procedures described previously [16, 19]. The 16S rRNA gene sequence [1477 nucleotides (nt)] of strain B138\(^\text{T}\) was aligned using MEGA version 6 software [20] against the corresponding sequences of closely related type strains retrieved from the GenBank database using the EzTaxon-e server [21].

Phylogenetic trees were inferred by using the maximum-likelihood [22], maximum-parsimony [23] and neighbour-joining [24] tree-making algorithms drawn from the MEGA 6 package [20]; an evolutionary distance matrix for the neighbour-joining analysis was generated using the Jukes and Cantor [25] model. The appropriate nucleotide substitutions for the maximum-likelihood analysis were selected by the Bayesian Information Criterion (BIC) using MEGA 6 software and found to follow the Tamura-Nei parameter model (TN93+G+I; [26]). The topologies of the evolutionary trees were evaluated by a bootstrap analysis [27] of the neighbour-joining method based on a 1000 replicates using MEGA 6 software.

DNA–DNA relatedness values (\(\Delta Tm\)) between isolate B138\(^\text{T}\) and its nearest phylogenetic neighbours were determined using a fluorimetric method [28]. Genomic DNA was extracted according to a modified protocol of Van Soolingen et al. [18] from cultures growing in TSA for 7 d at 28 °C. The optimum temperature for renaturation (\(Tor\)) was calculated using \(Tor=0.51\ (%GC)+47\). The melting temperatures (\(Tm\)) at which 50% of the initial double stranded DNA denatured into single-stranded DNA for isolate B138\(^\text{T}\) genomic DNA and the isolate B138\(^\text{T}\)/*W. serinendens* DSM 45037\(^\text{T}\) and isolate B138\(^\text{T}/*W. deligens* DSM 44902\(^\text{a}\) hybrid DNA preparations were compared and the differences (\(\Delta Tm\)) calculated. The DNA G+C content (mol%) of isolate B138\(^\text{T}\) was estimated using the thermal denaturation method, as described by Gonzalez and Saiz-Jimenez [29]. DNA–DNA hybridizations were run twice with three replicates each.

Morphological and physiological characteristics were investigated by using glucose-yeast extract agar (GYEA) [30] for 7 days at 28 °C as the basal medium [31]. *W. serinendens* DSM 45037\(^\text{T}\) and *W. deligens* DSM 44902\(^\text{a}\) were used as standard controls. The temperature for growth was determined at 10, 28, 37 and 45 °C and at pH values of 4, 5, 9 and 10. NaCl tolerance for growth was tested with 1, 3, 5 and 7 % (w/v) NaCl added into the basal medium. Cell morphology and motility were observed by using phase-contrast and transmission electron microscopy, using cells from exponentially growing cultures, after incubation on GYEA for 3 days at 30 °C. For the latter, cells were negatively stained with 2 % (v/v) phosphotungstic acid, and after air drying, grids were examined and observed with a transmission electron microscope (model LEO 906; Zeiss) [32]. Oxidase and catalase activities, degradation ability and utilization of carbohydrates were determined according to the methods described by Kim [31]. Other physiological and biochemical properties were tested by using the API ZYM kit (bioMérieux) according to the manufacturer’s recommendations and using *W. serinendens* DSM 45037\(^\text{T}\), *W. deligens* DSM 44902\(^\text{a}\) and *W. maris* DSM 44693\(^\text{a}\) for comparison.

The isolate was examined to establish whether it had a chemotaxonomic profile typical of strains of species of the genus *Williamsia*. Standard procedures were used to determine the isomers of diaminopimelic acid present [33], the acyl type of myein [34], the menaquinone and polar lipids profiles [35], the whole-organism sugars [36] and the presence of mycolic acids [37] in strain B138\(^\text{T}\). Biomass for fatty acid analysis of the isolate and type strains of species of the genus *Williamsia* were harvested from Trypticase Soy Agar (TSA; Oxoid), which had been incubated at 30 °C for three days. Fatty acids extracted from isolate B138\(^\text{T}\) were methylated, separated by gas chromatography (Agilent 6890 GC) and analyzed by using the standard Sherlock Microbial Identification (MIDI) system, ACTINO version 5 [38]. *W. deligens* DSM 44902\(^\text{a}\), *W. serinendens* DSM 45037\(^\text{T}\) and *W. maris* DSM 44693\(^\text{a}\) were used as standard controls.

The analysis comparing 16S rRNA revealed that isolate B138\(^\text{T}\) is most closely affiliated with type strains of the genus *Williamsia*. Isolate B138\(^\text{T}\) shared its highest 16S rRNA similarity with the type strains of *W. serinendens*, *W. deligens* and *W. maris*: 99.0 %, 99.0 and 97.5 %, respectively. These values corresponded to 15–36 nt differences at 1477 sites. The corresponding 16S rRNA similarities with the remaining type
The isolate was found to contain meso-diaminopimelic acid as the diagnostic diamino acid in the peptidoglycan, N-glycolated muramic acid, and major amounts of diphosphatidyglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylinositol-dimannoside (Fig. S3). The predominant isoprenologue was dehydrogenated menaquinone with nine isoprene units [MK-9(H)]. The cellular fatty acid profile of the isolate contained major proportions (>10%) of C₁₅:0 (17.1%), C₁₈:0 10-methyl (24.8%) and C₁₈:1 ω₉c (14.3%) (Table 1).

Table 1. Major cellular fatty acid composition of strain B138ᵀ and of type strains of species of the genus *Williamsia*

<table>
<thead>
<tr>
<th>FAMEs</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁₄:0</td>
<td>6.0</td>
<td>6.1</td>
<td>6.1</td>
<td>10.2</td>
</tr>
<tr>
<td>C₁₅:0 ω₅c</td>
<td>1.5</td>
<td>0.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Summed feature 3*</td>
<td>11.9</td>
<td>14.4</td>
<td>15.2</td>
<td>10.6</td>
</tr>
<tr>
<td>C₁₆:0</td>
<td>17.1</td>
<td>20.9</td>
<td>20.3</td>
<td>23.7</td>
</tr>
<tr>
<td>C₁₇:0 ω₈c</td>
<td>7.0</td>
<td>1.9</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C₁₇:0</td>
<td>5.9</td>
<td>1.6</td>
<td>–</td>
<td>1.4</td>
</tr>
<tr>
<td>10-Methyl C₁₇:0</td>
<td>4.7</td>
<td>1.5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C₁₈:0</td>
<td>6.8</td>
<td>1.6.9</td>
<td>10.1</td>
<td>12.2</td>
</tr>
<tr>
<td>C₁₈:1 ω₉c</td>
<td>14.3</td>
<td>20.1</td>
<td>23.3</td>
<td>19.5</td>
</tr>
<tr>
<td>10-Methyl C₁₈:0</td>
<td>24.8</td>
<td>25.0</td>
<td>23.7</td>
<td>22.4</td>
</tr>
</tbody>
</table>

*Summed fatty acids (Feature3: C₁₆:1 ω₇c + iso-C₁₆:1 ω₇c 2-OH) that cannot be separated by GLC with the MIDI System.

The DNA base composition of isolate B138ᵀ was estimated as 73.3 mol%. The extent of DNA-DNA relatedness between isolate B138ᵀ and the type strains of *W. serinedens* and *W. deligens* were 64.4±4.1 and 62.3±4.1, respectively. The values are below the 70% cut-off point recommended for the assignment of prokaryotic strains to the same genomic species [39].

Physiological and biochemical characteristics are given in Table 2 and in the species description. Isolate B138ᵀ can be readily distinguished from the type strain of *W. serinedens*, its closest phylogenetic neighbour, by its ability to assimilate ribose and to degrade starch, and by its inability to use oxalic acid, lactic acid and uric acid as the sole carbon source.
Mycolic acids were presented and the chromatographic pattern was almost identical to the mycolic acids of W. serinedens DSM 45037<sup>T</sup> and W. maris DSM 44693<sup>T</sup>. As the reference strains contain short chain mycolic acids with 50–56 carbon atoms [1], it can be inferred that strain B138<sup>T</sup> produced mycolic acids of similar size (Fig. S4). All of these chemical properties support the assignment of the isolate to the genus Williamsia [1, 40].

It is also evident from all of the genotypic and phenotypic data that isolate B138 can be distinguished readily from its phylogenetic neighbours. It is, therefore, proposed that strain B138<sup>T</sup> should be recognized as a novel species of the genus Williamsia, with the suggested name, Williamsia spongiae sp. nov.

**DESCRIPTION OF WILLIAMSIA SPONGIAE SP. NOV.**

Williamsia spongiae (spon’gi.ae. L. gen. n. spongiae of a sponge).

Aerobic, Gram-stain-positive, non-sporulating, non-motive, rod-shaped cells (0.6–0.8×2.2–2.6 µm) arranged singly, in pairs or in clusters. Colonies are orange, smooth and regular on ISP 2 agar. The optimum temperature for growth is 30 °C, but it also grows at temperatures from 10 to 45 °C. Grows at a pH range from 4 to 10. Grows in basal medium with 1 to 7 % (w/v) NaCl added. Negative for oxidase and catalase positive. Casein, starch, cellulose, guanine, hypoxanthine, xanthine, xylan, Tween 20, 40 and 60 are hydrolyzed, but not Tween 80 or gelatin. Grows in the presence of nalidixic acid, penicilin G and Novobiocin (10 µg ml<sup>–1</sup>). Produce weak response for α-chymotrypsin, alkaline phosphatase, β-glucosidase and cystine arylamidase and positive response for leucine arylamidase, naphthol AS-BI phosphohydrolase, esterase (C4), esterase lipase (C8), lipase (C14), acid phosphatase and α-glucosidase (API ZYM tests). Grows on D-fructose, D-ribose, fructose and malic acid, but not on glycerol, meso-inositol, rhamnose, mannitol, D-sorbitol, uric acid, lactic acid, D-mannose, D-galactose, cellobiose, lactose, raffinose, L-arabinose, adonitol, meso-erithritol or oxalic acid as the sole carbon and energy source.

**Table 2.** Comparison of the biological characteristics distinguishing strain B138<sup>T</sup> from other members of the genus Williamsia

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Orange</td>
<td>Pink-orange</td>
<td>Orange</td>
<td>Orange</td>
</tr>
<tr>
<td>ZYM-API</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>w</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucoisidase</td>
<td>w</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl-beta-glucosaminidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Leucine arylamidase</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>Assimilation of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Ribose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Manitol</td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>Casein</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Uric acid</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>NA</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Xanthine</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Additional phenotypic properties are cited in the text or in Tables 1 and 2. The predominant menaquinone is MK-9 (H₂) and the polar lipid profile comprises diphasitidyglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositoldimannoside and phosphatidylinositol. The diagnostic diamino acid in the cell walls is meso-diaminopimelic acid and for muramic acid it is N-glycolated. Arabinose and galactose were detected as the whole-cell sugars.

The type strain is B138T (=CBMAI 1094T=DSM 46676T) and it was isolated from the marine sponge, Amphimedon viridis, which was collected from São Paulo, Brazil. The genomic DNA G+C content of the type strain is 73.3 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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