Blastococcus capsensis sp. nov., isolated from an archaeological Roman pool and emended description of the genus Blastococcus, B. aggregatus, B. saxobsidens, B. jejuensis and B. endophyticus

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A novel Gram-reaction-positive actinobacterium, designated BMG 804T, was isolated from an archaeological Roman pool located in Gafsa, Tunisia. The strain grew as dry bright orange colonies at 30°C and pH 6.0–8.0. It contained meso-diaminopimelic acid in the cell wall. The whole-cell sugars consisted of glucose, rhamnose and ribose. Polar lipids present were diphasphatidylglycerol, phosphatidylethanolamine, phosphatidyldcholine, phosphatidylinositol, an unidentified glycolipid and two unidentified phospholipids. MK-9(H4) was the predominant menaquinone. The fatty acid profile contained major amounts (>5 %) of C17:1ω8c, C18:1ω7c, iso-C15:0, iso-C16:0 and iso-C15:0 2-OH. The 16S rRNA gene sequence of BMG 804T showed 99.4 % as highest sequence similarity with Blastococcus saxobsidens. DNA–DNA hybridization between strain BMG 804T and B. saxobsidens DSM 44509T was 48.6±6.6 %. The G+C content of the DNA was 73.7 mol%. On the basis of the phenotypic and genotypic characteristics, including DNA–DNA hybridization results, BMG 804T (=DSM 46835T=CECT 8876T) is proposed as the type strain of a novel species Blastococcus capsensis sp. nov. Emended descriptions of the genus Blastococcus and the species Blastococcus aggregatus, B. saxobsidens, Blastococcus jejuensis and Blastococcus endophyticus are also proposed.

The genus Blastococcus (Ahrens & Moll, 1970; Urzi et al., 2004; Lee, 2006) accommodates actinobacteria, often species forming buds or rods, giving the name of the genus; Normand et al. (2014). The genus Blastococcus is classified together with Geodermatophilus and Modestobacter in the family Geodermatophilaceae (Normand, 2006; Normand et al., 2014) of the order Geodermatophilales (Sen et al., 2014). There are four species of the genus Blastococcus with validly published names: Blastococcus aggregatus (Ahrens & Moll, 1970; Urzi et al., 2004), Blastococcus saxobsidens (Urzi et al., 2004), Blastococcus jejuensis (Lee, 2006) and Blastococcus endophyticus (Zhu et al., 2013). These were, respectively, isolated from sediment of Station Breitengrund at a depth of 20 m in the Western Baltic Sea, Marble Eleusys an archaeological site in Greece, sand sediment of a Korean beach in Jeju and from healthy leaves of Camptotheca acuminata, a traditional Chinese medicinal plant. Recently a non-validly named species, Candidatus Blastococcus massiliensis has been reported from a single anorexia nervosa stool sample (Pfleiderer et al., 2013). The complete genome sequence (Chouaia et al., 2012) and
proteome (Sghaier et al., 2016) have been obtained for *B. sax-
obsidens* strain DD2, an isolate obtained from the interior of
monument stone (Urzi et al., 2001). In this study, an isolate
representing a novel species was isolated from a limestone
sample from the wall rock of a Roman pool in Gafsa, Tunisia.

Strain BMG 804\(^T\) was isolated from dust collected from the
interior of a limestone rock in a wall of an archaeological
Roman pool located in Gafsa, southwest Tunisia (34° 25' N
008° 47' E, depth 285 m). Samples were suspended (ratio
1:10 w/v) in physiological saline solution (0.85 % NaCl),
shaken for 1 h (200 r.p.m.) at 26°C and kept overnight at
4°C and then shaken again for 2 h before being streaked out
on Luedemann medium (DSMZ medium 877) (Luedem-
mann, 1968). Plates were supplemented with cycloheximide
(100 µg ml\(^{-1}\)) and incubated at 28°C for 2 weeks (Giongo
et al., 2013). The isolate was sub-cultured four times on
GYM Streptomycyes medium (DSMZ medium 65). The pure
culture was preserved in a glycerol suspension (35 %, w/v)
at −80°C. BMG 804\(^T\) was grown on GYM Streptomycyes,
PYGV (DSMZ medium 621), R2A and Luedemann media
for examination of morphological and physiological
characteristics.

Morphological characteristics of BMG 804\(^T\) were examined
using an optical microscope (AxioScope A1; Zeiss) with a
100× magnification and phase-contrast illumination and
with a field-emission scanning electron microscope (FE-SEM
Merlin; Zeiss). Gram reaction was carried out by the KOH
test described by Gregersen (1978). Oxidase activity was deter-
mined using a 1 % (w/v) solution of N,N,N',N'-tetramethyl-
p-phenylenediamine (Sigma-Aldrich). A positive test was
defined by the development of a blue-purple colour after
applying biomass to the filter paper (Kovacs, 1956). Catalase
production was tested on slides by the formation of bubbles
after mixing a fresh bacterial suspension with a drop of 3 %
hydrogen peroxide solution. Enzymatic activities were tested
using the API ZYM system according to the manufacturer’s
instructions (bioMérieux). Phenotypic fingerprints of BMG
804\(^T\) and the reference strains *B. aggregatus* DSM 4725\(^T\),
*Streptomyces* DSM 44509\(^T\), *B. jeuniensis* DSM 19597\(^T\) and
*B. endophyticus* DSM 45413\(^T\) were comparatively analysed
in duplicate using GEN III Microplates in an Omnilog device
(Biolog) at 28°C. Cell suspensions were prepared in a viscous
inoculating fluid (IFC) provided by the manufacturer at 83 %
transmittance (T) for BMG 804\(^T\) and at 95 % T for reference
strains. Data were exported and analysed using the opm pack-
age for R v.1.0.6 (Vaas et al., 2012, 2013). Reactions with
different results between the two replicates were regarded as
ambiguous. Growth of strains after 7 days was assessed at tem-
peratures ranging from 5 to 60°C in steps of 5°C on GYM
Streptomycyes medium. The ability of the strain to grow on
modified ISP2 medium (Shirling & Gottlieb, 1966) at pH val-
ues adjusted from 4.0 to 12.5 (in steps of 0.5 pH units)
by adding NaOH or HCl (since the use of a buffer system inhib-
ited bacterial growth), was also tested. Growth of BMG 804\(^T\)
in the presence of 0–10 % NaCl (w/v) was determined on
yeast extract–malt extract medium (ISP 2). Additionally, pro-
duction of indole, nitrate reduction and hydrolysis of tyrosine
(Gordon & Smith, 1955), casein, starch, xanthine, hypoxan-
thine (Montero-Calasanz et al., 2012), gelatin (Clarke, 1953) and
easculin (Swan, 1954) were tested.

For chemotaxonomic investigations, BMG 804\(^T\) was culti-
vated in GYM Streptomycyes broth (DSMZ medium 65),
shaken (180 r.p.m.) at 28°C for 7 days. Whole-cell amino
acids and sugars were prepared according to the methods
of Lechevalier & Lechevalier (1970), followed by thin layer chro-
atography (TLC) analysis (Staneck & Roberts, 1974). The
composition of peptidoglycan hydrolysates (6 M HCl, 100°C
for 16 h) was analysed by TLC on cellulose plates as described
by Schleifer & Kandler (1972). Menaquinones (MK) were
extracted using the procedures of Collins et al. (1977) and
analysed by high-performance liquid chromatography
(HPLC) (Kroppenstedt, 1982). Polar lipids were extracted from
freeze-dried cell material and then identified by two-
dimensional TLC as described by Minnikin et al. (1984) with
modifications proposed by Kroppenstedt & Goodfellow
(2006). Moreover, choline-containing lipids were detected by
spraying with Dragendorff’s reagent (Merck Millipore,
102035) (Tindall, 1990). Fatty acid extraction for BMG 804\(^T\)
was carried out in duplicate from 16-days-old culture growing
at 20°C on PYGV agar plates. Fatty acid methyl esters were
obtained from biomass taken from plates by saponification,
methylation and extraction. Analysis was performed using the
Microbial Identification System (MIDI) Sherlock Version 6.1
(method TSBA40, ACTIN6 database) and peaks were inte-
grated and identified automatically by the Microbial Identifi-
cation software package as described by Sasser (1990). The
annotation of the fatty acids in the ACTIN6 peak naming
table is consistent with IUPAC nomenclature (i.e. double
bond positions identified with reference to the carboxyl group
of the fatty acid), but for consistency with other publications
this has been altered to numbering from the aliphatic end of
the molecule (i.e. C\(_{16:1}\) CIS 9 becomes C\(_{16:1\Delta9c}\) and C\(_{17:1}\)
CIS 9 becomes C\(_{17:1\Delta5c}\)). All chemotaxonomic analyses were
conducted under standardized conditions with strain BMG
804\(^T\) and cultures of the same set of reference strains as listed
above for the phenotypic fingerprints.

DNA extraction, PCR-amplification and sequencing of the
16S rRNA gene were performed as previously described
(Rainey et al., 1996). The identity of BMG 804\(^T\) was deter-
mined based on its 16S rRNA sequence using EzTaxon
(http://eztaxon-e.ezbiocloud.net) (Kim et al., 2012) and RDP-
II servers (Maidak et al., 2001). Phylogenetic analyses and
rooting of the inferred tree were conducted as previously
described (Montero-Calasanz et al., 2013) using the DSMZ
phylogenomics pipeline (Meier-Kolthoff et al., 2014) adapted
to single genes integrated in the GGDC web server (Meier-
Kolthoff et al., 2013a) available at http://ggdc.dsmz.de/. Pair-
wise similarities were calculated as recommended by
Meier-Kolthoff et al. (2013b) for the 16S rRNA gene available
via the GGDC web server. For DNA–DNA hybridization tests,
cells of BMG 804\(^T\) and *Blastococcus saxobsidens* DSM 44509\(^T\)
were disrupted by using a Constant Systems TS 0.75 kW (IUL
Instruments). DNA in the crude lysate was purified by chro-
atography on hydroxyapatite as described by Cashion et al.
modifications described by Huss et al. (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostat-regulated 6 × 6 multicolor changer and a temperature controller with in situ temperature probe (Varian). The G+C content (mol%) of the genomic DNA was determined by using the HPLC method according to the protocol of Mesbah et al. (1989).

BMG 804 T was a Gram-reaction-positive actinobacterium. Colonies were bright orange, opaque with dry surfaces and regular margins. Cell morphology was characterized by non-motile cocci in pairs and tetrads or tending to form aggregates as described for members of the genus Blastococcus (Lee, 2006). However bud formations, germ tubes or multicell changer and a temperature controller with in situ temperature probe (Varian). The G+C content (mol%) of the genomic DNA was determined by using the HPLC method according to the protocol of Mesbah et al. (1989).

Whole-cell hydrolysates of BMG 804 T contained mesodiaminopimelic acid (cell wall type III) (Lechevalier & Lechevalier, 1970) which is consistent with the affiliation to the family Geodermatophilaceae (Normand & Benson, 2012). Whole-cell sugar analysis of BMG 804 T revealed glucose, rhamnose and ribose as diagnostic sugars. The presence of galactose has been described as a taxonomic feature of the genus Blastococcus by Lee (2006), nevertheless, as shown in the Table 1, its presence was not consistent in the genus. It was not detected either in the whole-cell sugars of BMG 804 T, B. aggregatus DSM 4725 T or B. jejunis DSM 19597 T. Similarly, arabinose was only revealed in the whole-cell extracts of B. aggregatus DSM 4725 T and B. endophyticus DSM 45413 T (at trace level). The presence of xylose in the extracts of B. jejunis represents a diagnostic feature of the species.

The predominant menaquinone was MK-9(H4) (60.8%) in accordance with data reported for the members of the family Geodermatophilaceae (Normand, 2006; Normand et al., 2014) but MK-9 (29.5%) and MK-9(H2) (5.3%) were also detected. MK-9 (7.4%) and MK-9(H2) (1.1%) were likewise observed in B. saxobsidens DSM 44509 T, whereas MK-9 was only detected in B. jejunis DSM 19597 T (32.3 %) and B. endophyticus DSM 45413 T (9.4%). Menaquinone MK-8(H4) was, in addition, part of the menaquinone profiles of B. saxobsidens DSM 44509 T (7.1%) and B. endophyticus DSM 45413 T (4.4%) representing a primary menaquinone in B. aggregatus DSM 4725 T (63.6 %). MK-9(H4) was also identified as a minor component (2.5 %) in B. saxobsidens DSM 44509 T. The presence of menaquinones different from MK-9(H4) and MK-9 has already been described by Urzì et al. (2004) for isolates of B. saxobsidens and in the original description of B. endophyticus by Zhu et al. (2013) without emendation of the genus description for inclusion of additional menaquinones. Moreover neither MK-7(H2) in B. saxobsidens DSM 44509 T nor MK-8 in B. endophyticus DSM 45413 T was detected in our studies. Major fatty acids (>5%) were C17:1ω8c (22.1±4.7%), C16:ω7c (17.7±0.1%), iso-C15:0 (13.8±2.0%), iso-C16:0 (11.6±1.6%) and iso-C16:1H (5.9±0.7%), consistent with the major fatty acids described for members of the genus Blastococcus. Diposphatidyglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylycholine (PC) and phosphatidylinositol (PI) were the major polar lipids of BMG 804 T with their chromatographic mobility documented in Fig. 1a. In addition, an unidentified glycolipid (GL) and two unidentified phospholipids (PL1–2) were also encountered in low concentrations. This is in accordance with the chromatographic profiles observed in this study for the references strains (Fig. 1b–e) and those published by Lee (2006) and Zhu et al. (2013), although the absence of phosphatidyglycerol (present in the original descriptions of B. saxobsidens and B. jejunis) should be noted. Such absence has already been described for other members of the family Geodermatophilaceae (Montoro-Calasanz et al., 2014, 2015; Hezibri et al., 2015a, b, 2016). In addition, it is worth mentioning that the two unidentified phospholipids (PL1 and PL2) observed in BMG 804 T were also detected in the polar lipids profile of B. saxobsidens (Fig. 1e). On the other hand, a characteristic unidentified glycosylphospholipid (GPL) was observed in the chromatographic profiles of B. aggregatus, B. saxobsidens and B. jejunis, switching to an unidentified GL in the profiles of B. endophyticus and BMG 804 T.

Based on almost complete (1329 bp) 16S rRNA gene sequences, BMG 804 T clustered together with type strains of all members of the genus Blastococcus within the same phylogenetic group based on maximum-likelihood inference (Fig. 2). Meier-Kolthoff et al. (2013b) linked an Actinobacteria-specific 16S rRNA threshold of 99.0%, with 1.0% as maximum probability of error, to DNA–DNA hybridization (DDH) values above the 70% threshold required to assign a given strain to a new species (Wayne et al., 1987). Based on its 16S rRNA gene sequence similarities being 99.4% with B. saxobsidens DSM 44509 T, 98.4% with B. endophyticus DSM 45413 T, 98.0% with B. aggregatus DSM 4725 T and 97.9% with B. jejunis DSM 19597 T, only the DDH value with the type strain of B. saxobsidens was determined and corresponded to 48.6±6.6%. The G+C content of the DNA was 73.7 mol%.

The 16S rRNA gene sequence and DNA–DNA relatedness together with some physiological and chemotaxonomic differences (Table 1) clearly warrant the proposal of a novel species to accommodate BMG 804 T, for which the name Blastococcus capsensis sp. nov. is proposed.

Based on a review of the literature and new data obtained in this study, emended descriptions of the genus Blastococcus and the species B. aggregatus, B. saxobsidens, B. jejunis and B. endophyticus were also provided.
Table 1. Differential phenotypic characteristics of strain BMG 804<sup>T</sup> and the type strains of other species of the genus *Blastococcus*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell shape</strong></td>
<td>Cocci</td>
<td>Cocci, rods, vibrios&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Cocci†</td>
<td>Cocci, rods‡</td>
<td>Cocci&lt;sup&gt;++&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Bud formation</strong></td>
<td>–</td>
<td>+*</td>
<td>–†</td>
<td>–‡</td>
<td>–</td>
</tr>
<tr>
<td><strong>Germ tube</strong></td>
<td>–</td>
<td>+*</td>
<td>–†</td>
<td>–‡</td>
<td>–</td>
</tr>
<tr>
<td><strong>Motility</strong></td>
<td>–</td>
<td>+*</td>
<td>–†</td>
<td>–‡</td>
<td>+*</td>
</tr>
<tr>
<td><strong>Pigmentation</strong></td>
<td>Bright orange</td>
<td>Pink</td>
<td>White to pink to black</td>
<td>Apricot</td>
<td>Pink to orange</td>
</tr>
<tr>
<td><strong>Temperature range for growth (°C)</strong></td>
<td>20–37</td>
<td>10–40§</td>
<td>10–45†</td>
<td>10–37‡</td>
<td>20–37*</td>
</tr>
<tr>
<td><strong>Enzymatic activities</strong></td>
<td>Oxidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td><strong>Oxidation of:</strong></td>
<td>Dextrin</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>β-Gentiobiose</strong></td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Methyl β-D-glucoside</strong></td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>–</td>
<td>±</td>
</tr>
<tr>
<td><strong>D-Salicylic acid</strong></td>
<td>–</td>
<td>–</td>
<td>±</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td><strong>N-Acetyl-D-glucosamine</strong></td>
<td>–</td>
<td>+</td>
<td>±</td>
<td>–</td>
<td>±</td>
</tr>
<tr>
<td><strong>N-Acetyl-neuraminic acid</strong></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>±</td>
</tr>
<tr>
<td><strong>D-Sorbitol</strong></td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>D-Mannitol</strong></td>
<td>–</td>
<td>–</td>
<td>±</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>D-Aspartic acid</strong></td>
<td>–</td>
<td>–</td>
<td>±</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Gelatin</strong></td>
<td>–</td>
<td>+</td>
<td>±</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>L-Alanine</strong></td>
<td>–</td>
<td>+</td>
<td>±</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>L-Arginine</strong></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>L-Aspartic acid</strong></td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td><strong>Pectin</strong></td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>D-Galacturonic acid</strong></td>
<td>+</td>
<td>–</td>
<td>±</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>D-Gluconic acid</strong></td>
<td>+</td>
<td>–</td>
<td>±</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td><strong>Mucic acid</strong></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>D-Saccharic acid</strong></td>
<td>–</td>
<td>–</td>
<td>±</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td><strong>β-Hydroxybutyric acid</strong></td>
<td>–</td>
<td>–</td>
<td>±</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>α-Ketobutyric acid</strong></td>
<td>–</td>
<td>±</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>Butyric acid</strong></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>±</td>
</tr>
<tr>
<td><strong>Sodium bromate</strong></td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>Phospholipids</strong></td>
<td>DPG, PE, PC, PI, 2PL</td>
<td>DPG, PE, PC, PI, GPL PC</td>
<td>DPG, PE, PC, PI, GPL PC</td>
<td>DPG, PC, PE, PI, GPL PC</td>
<td>DPG, PC, PE, PC, PI, GPL PC</td>
</tr>
<tr>
<td><strong>Diagnostic sugar</strong></td>
<td>Glucose, rhamnose, ribose</td>
<td>Ribose, arabinose, mannose, glucose</td>
<td>Glucose, galactose and traces of rhamnose, ribose, mannose and arabinose</td>
<td>Rhamnose, ribose, xylose, glucose and traces of mannose</td>
<td>Glucose, galactose and traces of ribose and mannose</td>
</tr>
<tr>
<td><strong>Menaquinones (MK)</strong></td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;), MK-9(H&lt;sub&gt;8&lt;/sub&gt;)</td>
<td>MK-8(H&lt;sub&gt;4&lt;/sub&gt;, MK-9(H&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;, MK-9(H&lt;sub&gt;8&lt;/sub&gt;)</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;, MK-9(H&lt;sub&gt;8&lt;/sub&gt;)</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;, MK-9(H&lt;sub&gt;2&lt;/sub&gt;)</td>
</tr>
<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>73.7</td>
<td>73.9*</td>
<td>71.6†</td>
<td>72.3‡</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Data taken from Urzi et al. (2004).
†Data taken from Zhu et al. (2013).
‡Data taken from Lee (2006).
||The components are listed in decreasing order of quantity.
¶Only components making up ≥1 % peak area ratio are shown.
Fig. 1. Polar lipids profiling of BMG 804\textsuperscript{+} (a), \textit{B. aggregatus} (b), \textit{B. endophyticus} (c), \textit{B. jejuensis} (d) and \textit{B. saxobsidens} (e) after separation by two-dimensional TLC using the solvents chloroform/methanol/water (65 : 25 : 4; by volume) in the first dimension and chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4; by volume) in the second one. Plates were sprayed with molybdatophosphoric acid (3.5 %; Merck) for detection of the total polar lipids. DPG, diphosphadidylglycerol; PE, phosphatidethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PME, phosphomethyletanolamine; GL, unidentified glycolipid; PL1–3, unidentified phospholipids; GPL, unidentified glycophospholipid; AL, aminolipid; L1–8, unidentified lipids. All data are from this study.
Emended description of the genus
Blastococcus Ahrens and Moll 1970
emend. Urzi et al. 2004 and Lee 2006

The properties are as given by Ahrens & Moll (1970) and emended by Urzi et al. (2004) and Lee (2006) with the following emendations regarding chemotaxonomy. The diagnostic diamino acid is, as previously described, meso-diaminopimelic acid. The basic polar lipids profile involves diphosphatidylglycerol, phosphatidylglycerol and phosphatidylglycolinositol. The presence of phosphatidylethanolamine and an unidentified glyco phospholipid (alternatively an unidentified glycolipid), is frequent. Phosphatidylethanolamine and three unidentified phospholipids may also be present depending on species. The characteristic whole-cell sugar pattern consists of ribose, glucose and mannose. The presence of arabinose, rhamnose, galactose and xylose is variable. The predominant menaquinone is usually MK-9(H4) but MK-8(H4) could appear as the primary menaquinone in some species. In addition MK-9 may also occur in significant amounts. MK-9 (H2), MK-9(H6) and MK-8 may also be present.

The type species is Blastococcus aggregatus.

Emended description of Blastococcus aggregatus
Ahrens and Moll 1970 emend. Urzi et al. 2004

The properties are as given in the species description by Ahrens & Moll (1970) and emended by Urzi et al. (2004) with the following emendation. In addition to diphosphatidylglycerol and phosphatidylglycolinositol, the polar lipids profile contains phosphatidylcholine and an unidentified glycolipid (the chromatographic mobility of which is documented in Fig. 1b). Phosphatidylglycerol, phosphatidylethanolamine and the two unidentified phospholipids listed by Urzi et al. (2004) are absent. The whole-cell sugars consist of ribose, arabinose, mannose and glucose. The predominant menaquinones are MK-8(H4) and MK-9(H4). MK-9 is absent.

The type strain is B15T=ATCC 25902T=DSM 4725T=JCM 12602T=NCIMB 1849T.

Emended description of Blastococcus endophyticus
Zhu et al. 2013

The properties are as given in the species description by Zhu et al. (2013) with the following emendation. In addition to diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and phosphatidylglycositol listed by Zhu et al. (2013) an unidentified glycolipid is present (the chromatographic mobility of which is documented in Fig. 1c). Phosphatidylglycerol, phosphatidylglycositolmannoside and the four unidentified phospholipids listed by Zhu et al. (2013) are absent. MK-9(H4) is the predominant menaquinone, but MK-9 and MK-8(H4) are present as minor components. MK-8 and MK-9(H6) are absent.
The type strain is YIM 68236<sup>T</sup> = CCTCC AA 209045<sup>T</sup> = DSM 45413<sup>T</sup> = KCTC 19998<sup>T</sup>.

**Emended description of Blastococcus jejuni**sis Lee 2006

The properties are as given in the species description by Lee (2006) with the following emendation. In addition to diphasphatidylglycerol, phosphatidylethanolamine, phosphatidylycholine, phosphatidylinositol and phosphatidylmethylethanolamine listed by Lee (2006), the polar lipids profile contains an unidentified glycolipid (the chromatographic mobility of which is documented in Fig. 1e). The whole-cell sugars consist of glucose, galactose and traces of mannose. Arabinose and galactose are not detected. MK-9(H<sub>4</sub>) and MK-9 are the predominant menaquinones.

The type strain is KST3-10<sup>T</sup> = DSM 19597<sup>T</sup> = JCM 15614<sup>T</sup> = KCCM 42251<sup>T</sup> = NRRL B-24440<sup>T</sup>.

**Emended description of Blastococcus saxobsidens** Urži et al. 2004

The properties are as given in the species description by Urži et al. (2004) with the following emendation. In addition to diphasphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol, the polar lipids profile contains phosphatidylcholine, three unidentified phospholipids and an unidentified glycolipid (the chromatographic mobility of which is documented in Fig. 1e). Phosphatidylglycerol, listed by Urži et al. (2004), is absent. The whole-cell sugars consist of glucose, galactose and traces of ribose and mannose. MK-9(H<sub>4</sub>) is the predominant menaquinone, but MK8 (H<sub>4</sub>), MK-9, MK-9(H<sub>4</sub>) and MK-9(H<sub>2</sub>) are present as minor components. MK7-(H<sub>4</sub>) is absent.

The type strain is BC444<sup>T</sup> = DSM 44509<sup>T</sup> = JCM 13239<sup>T</sup> = NRRL B-24246<sup>T</sup>.

**Description of Blastococcus capsensis sp. nov.**

*Blastococcus capsensis* (caps.en’sis, N.L. masc. adj. capsensis of or belonging to Capsa, the archaeological city now known as Gafsa located in southeast Tunisia, where the bacterium was collected).

Colonies are bright orange, opaque with dry surfaces and regular margins. Cells are Gram-reaction-positive, catalase and oxidase negative. Negative for aeruginosan degradation, indole production and casein, tyrosine, starch, gelatin, xanthine and hypoxanthine degradation. Nitrate is not reduced to nitrite. Temperature range for growth is 20–30 °C and pH range for growth is 5.5–9.0. NaCl is not needed for growth; the strain can grow in the presence of 1 % NaCl but not up to 4 % NaCl. The following enzymatic activities according to API ZYM strips are present: alkaline phosphatase, esterase lipase (C8), leucine arylamidase and α-glucosidase. According to the Biolog System, it oxidises D-maltose, D-trehalose, D-cellulbiose, sucrose, turanose, stachyose, D-raffinose, α-D-lactose, D-melibiose, methyl β-D-glucoside, D-glucose, D-mannose, D-fructose, D-galactose, 3-O-methyl-D-glucose, D-fucose, L-fucose, D-sorbitol, glycerol, D-glucose 6-phosphate, D-fructose 6-phosphate, L-aspartic acid, L-glutamic acid, L-pyroglutamic acid, pectin, D-galacturonic acid, D-glucuronic acid, glucuronamide, D-lactic acid methyl ester, α-ketoglutaric acid, D- and L-malic acid, malic acid, lithium chloride, β-hydroxybutyric acid, acetoacetic acid, propionic acid, acetic acid and aztreonam, but not dextrin, β-gentio-biose, D-salicin, N-acetyl-D-glucosamine, N-acetyl-neuraminic acid, inosine, sodium lactate, fusidic acid, D-serine, D-mannitol, D-arabitol, D-aspartic acid, treulacacid, rifamycin SV, minocycline, gelatin, L-alanine, L-arginine, L-serine, lincomycin, guanidine hydrochloride, niaprof, D-galactonic acid-gamma-lactone, mucic acid, D-saccharic acid, vancomycin, tetrazolium violet, tetrzolium blue, p-hydroxyphenylacetic acid, methyl pyruvate, L-lactic acid, bromosucinic acid, Tween 40, γ-aminobutyric acid, α-ketobutyric acid, sodium formate, butyric acid and sodium bromate. Predominant fatty acids are C<sub>17:0</sub>:ω6c, C<sub>16:0</sub>:ω7c, iso-C<sub>15:0</sub> and iso-C<sub>16:0</sub> and C<sub>16:0</sub> H<sub>4</sub>. The peptidoglycan in the cell wall contains meso-diaminopipelic acid. Glucose, rhamnose and ribose are the whole-cell sugars. MK-9(H<sub>4</sub>) is the predominant menaquinone, but MK-9 and MK-9(H<sub>2</sub>) are present as minor components. Polar lipids consist of diphasphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, an unidentified glycolipid and two unidentified phospholipids. The genomic DNA G+C content of the type strain is 73.7 mol%.

The INSDC accession number for the 16S rRNA gene sequences of the type strain BMG 804<sup>T</sup> (= DSM 46835<sup>T</sup> = CECT 8876<sup>T</sup>) is LN626274.

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**References**


