Thalassobacter stenotrophicus Macián et al. 2005 is a later synonym of Jannaschia cystaugens
Adachi et al. 2004, with emended description of the genus Thalassobacter

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The type strains of Jannaschia cystaugens (LMG 22015T) and Thalassobacter stenotrophicus
(CECT 5294T) were analysed by means of genomic DNA–DNA hybridization, comparison of
16S rRNA gene sequences and phenotypic properties determined under the same methodological
conditions. J. cystaugens LMG 22015T showed DNA–DNA relatedness levels of 72 % when
hybridized with the genomic DNA of T. stenotrophicus CECT 5294T. Sequence comparisons
revealed that the 16S rRNA genes of the two strains had a similarity of 99.8 %. The cellular fatty
acid and polar lipid compositions of the two strains and their DNA mol% G + C contents were
almost identical. Bacteriochlorophyll a (Bchl a) and polyhydroxybutyrate were produced by
both strains under the same culture conditions. Their closest phylogenetic neighbours were
Jannaschia helgolandensis and Jannaschia rubra; however, the low sequence similarity values
(95.7–95.9 %) and several important differences in phenotypic traits (ionic requirements, Bchl
a production and polar lipids) support the distinction between the genera Thalassobacter and
Jannaschia. Thus, we propose the unification of J. cystaugens (LMG 22015T) and T. stenotrophicus
(CECT 5294T) as Thalassobacter stenotrophicus (type strain, CECT 5294T = DSM 16310T).
An emended description of the genus Thalassobacter is also presented.

The continuous increase in the number of taxa within the Roseobacter group, or marine alpha-proteobacterial group,
in recent years has extended our knowledge of this essential and ubiquitous fraction of marine bacterioplankton. They
are currently recognized as belonging to the order ‘Rhodobacterales’, within the class ‘Alphaproteobacteria’ (Garrity
et al., 2004). An important number of genera/species belonging to this group are able to synthesize bacterio-
chlorophyll a (Bchl a) under aerobic conditions. These include Dinoroseobacter (Biebl et al., 2005), Roseivivax
(Suzuki et al., 1999b), Roseobacter (Shiba, 1991), Roseisalinus (Labrenz et al., 2005), Roseovarius (Labrenz et al.,
1999), Rubrimonas (Suzuki et al., 1999a), Staleyia (Labrenz et al., 2000) and Thalassobacter (Macián et al., 2005a).

The genus Thalassobacter was recently described as comprising a single species, Thalassobacter stenotrophicus,
represented by a single strain. The closest phylogenetic neighbour of Thalassobacter was the genus Jannaschia, from
which it could be distinguished by phenotypic traits and

phylogenetic distance. However, the recently described species Jannaschia cystaugens (Adachi et al., 2004) was not
included in the comparison, because the two descriptions originated from separate studies and the dates of publica-
tion overlapped. Nevertheless, when the 16S rRNA gene sequences of both organisms became available, it was noted
that T. stenotrophicus and J. cystaugens had almost identical sequences, in spite of the lack of close similarity in the
described phenotypes. Thus, we undertook a study to compare the two species by using a polyphasic approach, with
the aim of determining their relatedness by using the same methodology of phenotypic characterization and by com-
paring their genomic DNA similarity through DNA–DNA hybridization experiments. We also included in the study
the type strain (CECT 7023T) of the recently described species Roseisalinus antarcticus, in order to compare this
novel Bchl a-producing species with T. stenotrophicus.

The methods used for the phenotypic characterization and
DNA–DNA hybridization have been described in detail previously (Macián et al., 2005a, b). Phylogenetic analysis of
16S rRNA gene sequences was performed as described by Ludwig et al. (1998) using the software package ARB (Ludwig
et al., 2004). The strains used were T. stenotrophicus CECT
J. cystaugens LMG 22015<sup>T</sup> and R. antarcticus CECT 7023<sup>T</sup>. All three strains were routinely cultured on marine agar and in marine broth (MA and MB; Scharlab) at 22–25 °C, in the dark. The following phenotypic characteristics were determined in parallel: the ability to grow on MA diluted to 0·34, 0·85, 1·7 and 2·55 % (w/v) total salinity (dilution factors 0·1, 0·25, 0·50 and 0·75, respectively); the ability to grow on MA supplemented with NaCl to a total salinity of 6, 7, 8, 9 and 10 % (w/v); specific ionic requirements for Na<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup> and K<sup>+</sup> cations in salt-tolerance agar (STA) containing 1 % (w/v) tryptone, 0·3 % (w/v) yeast extract and 1·5 % (w/v) agar; the ability to grow at 4, 28, 37 and 40 °C on MA plates; extracellular hydrolytic activities on casein, starch, alginate, agar, Tween 80, lecithin and DNA; substrates used as sole carbon and energy sources for growth in basal medium agar (BMA; Baumann & Baumann, 1981); polyhydroxybutyrate (PHB) accumulation determined after staining cells grown on MA and on BMA plus acetate with Nile blue A (Smibert & Krieg, 1994); and Bchl<sub>a</sub> production determined in acetone extracts obtained from cells grown on MA, BMA plus lactate and BMA plus acetate using a Beckman DU-600 spectrophotometer, as described by Takaichi et al. (1991).

Cellular morphology was reported to be irregular rods for both J. cystaugens and T. stenotrophicus, but in the latter ovoid cells were also observed. The mode of division and absence of rosette formation were common to both strains.

The flagellar position of J. cystaugens was not stated in its original description, although the cells were reported as being motile. Very young cultures of T. stenotrophicus have one polar flagellum, but this trait was difficult to determine. Similar difficulties were found with J. cystaugens, which, in our hands, was non-motile, even in very young cultures, and showed no flagella.

Pigmentation was observed under various culture conditions, and was very similar in the two strains. It varied from light cream to dark brown, including salmon-pink and ochre-red colours. In general, the pigment was darker and more brown than pink in complex media such as casein- and lecithin-supplemented MA. It was poor in media that did not favour good growth, such as media with the highest or lowest salinity that supported growth (salinity range tests); however, deep pigmentation was also observed in some minimal media supporting poor growth. In any case, the pigmentation of the two strains was markedly similar when they were cultured under the same conditions and in sharp contrast to R. antarcticus, which always had a fainter pigmentation with different intensities of pink, but never ochre/brown.

The main differences in phenotypic traits between T. stenotrophicus and J. cystaugens, according to the original descriptions, are Bchl<sub>a</sub> production by T. stenotrophicus only and the occasional presence of white inclusion bodies (which could be interpreted as PHB) in J. cystaugens. Both characteristics were investigated in parallel in this study.

PHB production was determined by using Nile blue A staining and fluorescence microscopy, with cells grown on BMA plus acetate and on MA for 5 days. Escherichia coli 101 was used as a negative control and R. antarcticus CECT 7023<sup>T</sup> as a positive control. Both J. cystaugens and T. stenotrophicus showed the typical bright-orange fluorescence of PHB on BMA plus acetate-grown cells, but very few cells of either strain fluoresced when grown on MA. In contrast, PHB was produced by most cells of R. antarcticus when grown on MA. Therefore, we concluded that T. stenotrophicus is also able to produce PHB, although bright inclusions, a presumptive trait suggesting the presence of this polymer in bacterial cells, are not easily seen on wet mounts.

Bchl<sub>a</sub> production was determined as described by Takaichi et al. (1991) in acetone extracts of cells of strains CECT 5294<sup>T</sup> and LMG 22015<sup>T</sup> grown on MA, BMA plus acetate and BMA plus lactate for 7 days in the dark. All six culture extracts showed the typical absorbance peak of Bchl<sub>a</sub> at an identical position (772 nm), confirming the ability of J. cystaugens LMG 22015<sup>T</sup> to produce the photosynthetic pigment.

Identical results were also obtained with the two strains for the following phenotypic traits. They were unable to hydrolyse casein, starch, alginate, agar, lecithin, Tween 80 or DNA. The salinity range for growth was 1·7–7 % (w/v) total salinity. They were unable to grow in STA supplemented with the four salts tested (Na<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup> and K<sup>+</sup> cations), provided the inoculum was prepared in 2 % (w/v) NaCl. This indicates that both strains have complex ionic requirements. Strains LMG 22015<sup>T</sup> and CECT 5294<sup>T</sup> did not grow at 4 or 40 °C, but did grow at 28 and 37 °C. They utilized D-ribose, L-arabinose, D-glucose, D-fructose, D-glucosamine, D-glycerate, glycerol, D-mannitol, D-sorbitol, pyruvate, acetate, succinate, fumarate, malate, lactate, L-serine, L-arginine, L-glutamate, L-alanine and L-ornithine as carbon sources for growth on BMA supplemented with 0·01 % yeast extract. D-Trehalose, D-mannose, L-rhamnose, maltose, D-cellobiose, sucrose, lactose, D-melibiose, amygdalin, N-acetyl-D-glucosamine, D-glucaronate, m-inositol, glycine, L-leucine, L-threonine, L-tyrosine, 4-aminobutyrate, L-citrulline, L-histidine and putrescine did not support growth of any of the strains in the same media and under the same conditions. The instability of T. stenotrophicus CECT 5294<sup>T</sup> in the use of sole carbon sources is noteworthy, when the results obtained in this study were compared with those included in the original description. This instability, which was observed even when the same methodology was used at the same laboratory, could be due to the influence of factors such as the age of the cells used for the inoculation of BMA plates in different experiments, which was difficult to standardize. In any case, the carbon source profile obtained in this study for strain CECT 5294<sup>T</sup> was almost identical to that of strain LMG 22015<sup>T</sup>, with only differences in the use of L-aspartate being obtained.

The sequences used for the phylogenetic analysis were retrieved from the EMBL nucleotide sequence database. The
analysis showed a similarity of 99.8% between the 16S rRNA gene sequences of strains CECT 5294ᵀ and LMG 22015ᵀ, a value that clearly indicates that the two strains are related, at least at the genus level and probably also at the species level. To resolve the latter, DNA–DNA hybridization experiments with genomic DNA were performed in triplicate (Ziemke et al., 1998). A mean relatedness value of 72% was obtained, which is slightly above the suggested boundary for genomic species definition (Wayne et al., 1987).

In addition, we also observed a high concordance in both the mol% G+C content and cellular fatty acid compositions reported in the original descriptions (Adachi et al., 2004; Macián et al., 2005a). The G+C content of T. stenotrophicus CECT 5294ᵀ was 59.1 mol%, and those of J. cystaugens LMG 22015ᵀ and LMG 22016 were 59.1 and 59.2%, respectively. All three strains contained 67–78% of 18:1ω7c and minor amounts of 18:1ω7c 11-methyl (6.9–7.3%), 18:1ω9c (1.2–3.6%), 18:0 (2.6–2.8%), 10:0 3-OH (3.1–3.2%) and unknown ECL (2.9–3.6%). T. stenotrophicus CECT 5294ᵀ was reported to have a small amount (1.47%) of an undefined 19-carbon fatty acid, which could be 19:1ω6c or 19:0 cyclo. In the case of J. cystaugens, both strains completely lacked 19:0 cyclo, which was the characteristic second most abundant fatty acid of Jannaschia helgolandensis (20–25%) and was also present in Jannaschia rubra in smaller amounts (5.7%) (Wagner-Döbler et al., 2003; Macián et al., 2003b). Moreover, during the course of this work the polar lipid compositions of T. stenotrophicus CECT 5294ᵀ and J. cystaugens LMG 22015ᵀ were determined at the DSMZ. Polar lipid analyses were carried out by the Identification Service of the DSMZ and Dr B. J. Tindall (Braunschweig, Germany). The results reinforce the high similarity between the two strains, since their polar lipid compositions were essentially identical: phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, two unidentified phospholipids and one unidentified amino-lipid. T. stenotrophicus CECT 5294ᵀ also had minor amounts of a third unidentified phospholipid. The genus Jannaschia was described as containing phosphatidylethanolamine, which was absent in T. stenotrophicus CECT 5294ᵀ and J. cystaugens LMG 22015ᵀ. However, the distantly related Bchl a-producing species R. antarcticus (Labrenz et al., 2005) has a polar lipid composition that is virtually identical to those of T. stenotrophicus and J. cystaugens.

Transfer of Jannaschia cystaugens Adachi et al. 2004 to Thalassobacter stenotrophicus Macián et al. 2005

All the results reported here support the idea that T. stenotrophicus and J. cystaugens should be considered as representing a single species, as they were clearly separate in terms of genomic similarity from their closest phylogenetic neighbours and readily recognizable by their phenotypic characteristics, in agreement with the criteria of Wayne et al.

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**Fig. 1.** Unrooted phylogenetic consensus tree based on trees obtained using various datasets of 16S RNA gene sequences and alternative treeing methods (Ludwig et al., 1998). Multifurcations indicate branches for which their relative evolutionary order cannot be ascertained.
The names *Jannaschia cystaugens* Adachi *et al.* 2004 and *Thalassobacter stenotrophicus* Macián *et al.* 2005a can be considered as heterotypic synonyms. Following the general interpretation of Rules 15 and 17 of the *Bacteriological Code*, we conclude that the species should be given the name *Thalassobacter stenotrophicus*, with strain CECT 5294<sup>T</sup> (=DSM 16310<sup>T</sup>) as the type strain.

**Emended description of the genus Thalassobacter Macián *et al.* 2005**

The description is identical to that given by Macián *et al.* (2005a) with the following amendments. Positive for PHB accumulation. Major polar lipids are phosphatidylglycerol, diphosphatidylglycerol and phosphatidylcholine. Also contain two unidentified phospholipids and an unidentified aminolipid, but not phosphatidylethanolamine.

**Differentiation between *T. stenotrophicus* and *R. antarcticus***

The original description of *T. stenotrophicus* included a comparison of the new genus with its related genera. At the time of writing of that manuscript, the description of the new Bchl <i>a</i>-containing genus and species *R. antarcticus* (Labrenz *et al.*, 2005) was not available. Therefore, we included the type strain, CECT 7023<sup>T</sup>, in this study and we present here a comparison with *T. stenotrophicus*. The phenotypic traits as well as analysis of the 16S rRNA gene sequence (GenBank accession no. AJ605747) and G+C mol% content confirmed that, despite its ability to synthesize Bchl <i>a</i> under aerobic conditions and its very similar polar lipid composition, *Roseisalinus* is distantly related to *Thalassobacter*, showing only 92–93 % 16S rRNA gene sequence similarity. Moreover, when a phylogenetic analysis of members of the whole order *Rhodobacterales* was performed, the *Roseisalinus* sequence was seen to be more closely related to those of other genera such as *Oceanicola*, *Ketogulonicigenium* or *Loktanella*, whereas *Thalassobacter* is a neighbour of the genus *Jannaschia* (Fig. 1). On phenotypic grounds, the two genera could be clearly distinguished by growth at 10 % total salinity, ionic requirements (*Roseisalinus* being able to grow on STA supplemented with Na<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup> and K<sup>+</sup> cations, even when the inoculum was prepared in a 2 %, w/v, NaCl solution), rosette formation (observed only in

**Table 1. Phenotypic characteristics that differentiate *T. stenotrophicus* from *J. helgolandensis*, *J. rubra* and *R. antarcticus***

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigment on casein/MA</td>
<td>Deep brown</td>
<td>Deep brown</td>
<td>None</td>
<td>Red</td>
<td>Rose</td>
</tr>
<tr>
<td>Bchl &lt;i&gt;a&lt;/i&gt; production</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Rosette formation</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 8 % salinity</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Growth on STA plus Na&lt;sup&gt;+&lt;/sup&gt;, Mg&lt;sup&gt;2+&lt;/sup&gt;, Ca&lt;sup&gt;2+&lt;/sup&gt; and K&lt;sup&gt;+&lt;/sup&gt; cations</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Carbon sources:</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>D-Mannose</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>L-Rhamnose</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Sucrose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<tr>
<td>D-Cellobiose</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>D-Melibiose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<tr>
<td>m-Inositol</td>
<td>−</td>
<td>−</td>
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<td>+</td>
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<td>D-Sorbitol</td>
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<td>+</td>
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<td>−</td>
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<tr>
<td>D-Gluconate</td>
<td>+</td>
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<td>+</td>
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<td>−</td>
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<tr>
<td>L-Arginine</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>−</td>
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<td>Polar lipids:</td>
<td></td>
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<tr>
<td>Phosphatidylethanolamine</td>
<td>−</td>
<td>−</td>
<td>+&lt;sup&gt;6&lt;/sup&gt;</td>
<td>NA</td>
<td>−&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cellular fatty acids (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>&lt;1</td>
<td>−&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15±1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:1 o9c</td>
<td>1.2</td>
<td>3.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>−&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:1 o7c 11-methyl</td>
<td>7.0</td>
<td>7.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>G+C content (mol%)</td>
<td>59</td>
<td>59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>67&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Data from a, Adachi *et al.* (2004); b, Wagner-Döbler *et al.* (2003); c, Macián *et al.* (2005b); d, Labrenz *et al.* (2005).
$Roseisalinus$), cellular fatty acid composition, G+C content of the DNA and carbon sources that support growth (Table 1).

Acknowledgements

We are grateful to A. Camacho, Universitat de Valencia, for Bchl a spectrophotometry. This work was supported by project AGL-2002-04075-C02-C02 of the Spanish Ministerio de Ciencia y Tecnología. D.R.A. has a contract with the Universitat de Valencia under the Ramón y Cajal program (Ministerio de Ciencia y Tecnología).

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