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Genomic and genetic sequence information of strains assigned to the genus *Rhodopseudomonas* reveal the great heterogeneity of the group and identify strain *Rhodopseudomonas palustris* DSM 123^T as the authentic type strain of this species

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Abstract

The genus *Rhodopseudomonas*, containing purple nonsulfur photosynthetic Proteobacteria, has a number of strains that belong to different species, although many of them are collectively called *Rhodopseudomonas palustris*. The type species *R. palustris* and closely related species are the focus of this paper. The comparison of available genome sequences indicate that the following *Rhodopseudomonas* species are well recognized: *R. palustris* (strains ATH 2.1.6^T=DSM 123^T=NBRC 100419^T and BisB5), *Rhodopseudomonas rutila* (strains R1^T, DSM 126, CGA009, ATH 2.1.37, Eli 1980, ATCC 17001 and TIE1), *Rhodopseudomonas pentothenatexigens* JA575^T and *Rhodopseudomonas faecalis* JCM 11668^T. Other strains for which genome sequences are available are distinct from these four species. Evidence is presented that *R. palustris* strain ATH 2.1.6^T–KCM as obtained directly from the van Niel collection by one of us (T.E.M.) is identical to the DSMZ deposit DSM 123^T of ATH 2.1.6^T, but not to the deposit at ATCC 17001. The amino acid sequences of the cytochromes C2 and C556 from *R. palustris* strain ATH 2.1.6^T–KCM are in complete agreement with the translated genome sequences of *R. palustris* DSM 123^T. In addition, the 16S rRNA gene sequence of *R. palustris* NBRC 100419^T completely matches that of strain DSM 123^T. In conclusion, the type strain of *R. palustris* ATH 2.1.6^T is correctly represented by DSM 123^T and NBRC 100419^T. However, the deposit at ATCC 17001 has properties that do not conform with properties of authentic *R. palustris*, but rather indicate that this is a strain of *R. rutila*. The previously suggested assignment of the type strain of *R. palustris* DSM 123^T to the new species *R. pseudopalustris* was incorrect because strain DSM 123^T is the authentic type strain of *R. palustris*.

INTRODUCTION

Rhodopseudomonas palustris was the first *Rhodopseudomonas* species identified by van Niel in 1944 [1] and strain ATH 2.1.6^T was assigned as the neotype strain of this species by Pfennig and Trüper [2]. As *Rhodopseudomonas palustris* is the type species of the genus, it remained the reference species within this genus after several taxonomic revisions and transfers of species to other genera [3–5]. *Rhodopseudomonas rutila* R1^T (ATCC 33872^T) was described by Akiba *et al.* [6], and *Rhodopseudomonas faecalis* by Zhang *et al.* [7]. More recently, *Rhodopseudomonas thermotolerans* and *Rhodopseudomonas pentothenatexigens* were described [8]. The type strain of *R. palustris* DSM 123^T was transferred as type to the new species *Rhodopseudomonas pseudopalustris* [9].

In the context of this paper it is important to review the history of the type strain of *R. palustris* ATH 2.1.6^T (=DSM 123=NBRC 100419), which actually is a neotype as stated by Pfennig and Trüper in 1971 [2]. The strain was directly received from van Niel by ATCC (ATCC 17001), NCIMB (via S.R. Elsden) and by the Kamen/Cusanovich/Meyer laboratories. In this communication the ATH 2.1.6–KCM designation is used to indicate the origin of data from the strain in this lab. Derivatives of the strain were received by DSMZ (DSM 123) from ATCC 17001 and by NBRC (NBRC 100419) from NCIMB 8252.

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Abbreviations: ATCC, American Type Culture Collection; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen; NCIMB, National Collections of Industrial, Marine and Food Bacteria; NRBC, National Biological Resource Center.

NCIMB meanwhile had removed the strain from the catalogue of strains. Although DSMZ received the strain from the ATCC collection, the two deposits (ATCC 17001 and DSM 123^T) are obviously different organisms as known from earlier studies [10, 11]. The 16S rRNA gene sequences of the two derivatives of ATCC 17001^T, *R. palustris* NBRC 100419^T and DSM 123^T, completely match each other [11], but are different from that of ATCC 17001. As type strain derivatives of ATCC 17001 at NBRC and DSMZ were found to be identical and proof was given on the identity of DSM 123^T and the strain in the Kamen/Cusanovich/Meyer laboratories derived from the original source in the van Niel laboratory, of which also ATCC 17001 derived (see below), but different from ATCC 17001, it would appear that the strain at ATCC had changed properties and was mixed up with another bacterium, though after transfer of the derivatives to the other collections.

R. rutila was described as a new species by Akiba *et al.* [6] with strain R1^T assigned as type strain. In a later study by Hiraishi *et al.* [10] using DNA–DNA hybridization, the type strain of *R. rutila* R1^T was compared to the type strain of *R. palustris* as obtained from the American Type Culture Collection (ATCC 17001^T) and with a number of new isolates assigned at that time to *R. palustris*. The ATCC 17001 strain showed 78% DNA homology to *R. rutila* R1^T and the new isolates had between 78–91% homology to this reference strain. Unfortunately, the *R. palustris* type strain derived from other sources, DSM 123^T and NBRC 100419^T, was not included in this study. At that time, Hiraishi *et al.* [10] did not consider the possibility of a changed identity of strain ATCC 17001 as the type of *R. palustris*. Based on the identity of *R. rutila* R1 and strain ATCC 17001 they considered *R. rutila* as a heterotypic synonym of *R. palustris* [10].

The intrageneric relationship of the genus *Rhodopseudomonas* was also studied by Okamura *et al.* [11]. DNA–DNA hybridization experiments of this study included two sources of the reference type strains of *R. palustris*, strains ATCC 17001 and DSM 123^T, and demonstrated the lack of identity between the two type strains with only 25% homology [11]. This study also differentiated *R. rhenobacenis* DSM 12706^T and *R. faecalis* JCM 11668^T from both ATCC 17001 and DSM 123 with homology values between 18–30%, confirming their recognition as separate species. Also rRNA gene sequence comparison revealed clusters of strains one of which included *R. palustris* DSM 123^T and BisB5 and was clearly separate from another cluster with strains ATCC 17001^T, CGA009, ATCC 17003, ATCC 17007 and several new isolates. The two species *R. faecalis* g-c^T (JCM 11668^T) and *R. rhenobacensis* Rb^T (DSM 12706^T) (grouping with strain BisB18) were supported also by the rRNA gene phylogeny [11]. As expected, the gene sequences of the 16S rRNA genes showed complete identity between the two sources of the type strain of *R. palustris* DSM 123^T and NBRC 100419^T [11], but revealed clear differences from the ATCC 17001^T deposit. Unfortunately, this study did not include the type of *R. rutila* R1^T, which would have proven identity to ATCC 17001.

More recently, two new species of the genus *Rhodopseudomonas*, *Rhodopseudomonas harwoodiae* and *Rhodopseudomonas parapalustris*, were described and their differentiation from other species of the genus by DNA–DNA hybridization analysis demonstrated similarity values below 50% [9]. This study also supported the recognition of *R. rhenobacensis* and *R. faecalis* as separate species and pointed out differences at the species level between the two references of the type of *R. palustris*, strains DSM 123 and ATCC 17001. Unfortunately, this study did not include the type strain of *R. rutila* R1^T, but referred to ATCC 17001 of *R. palustris* as a reference. Most problematically, the transfer of the type strain of *R. palustris* ATH 2.1.6=DSM 123 (that matched the properties of the authentic original deposit, as demonstrated below) to the type of the new species *Rhodopseudomonas pseudopalustris* was also proposed by these authors [9].

It is necessary to correct this obvious mistake and reject the proposal which means that strain R1^T remains the type of *R. rutila* and all strains closely related to this strain shall be recognized as further representatives of this species. Also, evidence is given below that strain DSM 123^T as a direct derivative of ATCC 17001 should be maintained as type strain of *R. palustris*, as it is identical to other derivatives thereof and to a subculture of the original strain ATH 2.1.6 of van Niel.

METHODS

Origin of strains and sequences

A copy of the van Niels culture collection was obtained by one of us as living cultures (T.E.M.) between 1965 and 1970 and was kept frozen in glycerol in the Kamen/Cusanovich/Meyer laboratories over the years. *R. rutila* R1^T was obtained from T. Akiba.

The accession numbers of the genome sequences used in this study are: *Rhodopseudomonas faecalis* JCM11668^T (QJTI), *Rhodopseudomonas faecalis* MAG1 (SHOF01), *Rhodopseudomonas faecalis* JSC-3b (AYSU00000000.1), *Rhodopseudomonas faecalis* PSBS (JHAA01), *Rhodopseudomonas palustris* DSM 123^T (PRJEB16943), *Rhodopseudomonas palustris* BisB5 (CP000283.1), *Rhodopseudomonas pentothenatexigens* JA575^T (QRDT), *Rhodopseudomonas rutila* R1^T (QWVU01), *Rhodopseudomonas rutila* ATH 2.1.37 (QYYC01), *Rhodopseudomonas rutila* CGA009 (BX571963.1), *Rhodopseudomonas rutila* DSM 126 (NRS101), *Rhodopseudomonas rutila* ELI 1980 (CM001782.1), *Rhodopseudomonas rutila* PS3 (CP019966.1), *Rhodopseudomonas rutila* TIE-1 (ASM2044v1), *Rhodopseudomonas rutila* YSC3 (CP019967.1), *Rhodopseudomonas thermotolerans* JA576^T (QUMP), *Rhodopseudomonas* sp. ATH 2.1.18 (QYYD01), *Rhodopseudomonas* sp. 420L (LCZMO1), *Rhodopseudomonas* sp. AAP120 (LJIC01), *Rhodopseudomonas* sp. B29 (BADD01), *Rhodopseudomonas* sp. BAL398 (ASM93520v1), *Rhodopseudomonas* sp. BisA53 (CP000463.1), *Rhodopseudomonas* sp. BisB18 (CP000301.1), *Rhodopseudomonas* sp. DX-1 (CP002418.1, NC014834.1), *Rhodopseudomonas* sp. HaA2 (CP000250.1) and *Rhodopseudomonas* sp. XCP (QKQS01).

Methods of calculations

Average nucleotide identity (ANI) calculations were performed using Jspecies option ANIb [12], which uses a pairwise genome comparison algorithm to measure probability if two or more genomes belong to the same species. The compared genomes were either selected from the curated reference database GenomesDB (by JSpecies), or uploaded from GenBank for those not present in the JSpecies database.

The phylogenetic tree was built using the Codon Trees pipeline within PATRIC [13]. Codon Trees uses the amino acid and nucleotide sequences from a defined number of PATRIC's global Protein Families (PGFams) to build an alignment, and then generates a tree based on the differences within those selected sequences. Both the protein (amino acid) and gene (nucleotide) sequences are used for each of the selected genes from the PGFams. The number of single-copy genes from PGFams was set for 1000, and 872 were identified using the 26 *Rhodopseudomonas* strains in our analysis. Protein sequences were aligned using MUSCLE [14], and the nucleotide coding gene sequences were aligned using the Codon_align function of BioPython. A concatenated alignment of all proteins and nucleotides were written to a phylip formatted file, from which a partitions file for RaxML [15] was generated. Support values were generated using 100 rounds of the 'Rapid' bootstrapping option [16] of RaxML. The resulting Newick file was downloaded and visualized in iTOL [17].

RESULTS

In order to solve the taxonomic confusion and correct the proposals that have been based on wrong assumptions, we have compared the ANI values of a larger number of *Rhodopseudomonas* strains (with available genome sequences) and included available information on cytochrome sequences and a phylogenetic tree based on PATRIC's global protein families. The comparison included data from the type strain of *R. palustris* as obtained from van Niel's collection by one of us (T.E.M.; ATH 2.1.6–KCM) as well as from DSMZ (DSM 123), the type of *R. rutila* R1^T (obtained from T. Akiba), of *R. faecalis* JCM11668^T, *R. thermotolerans* JA576^T and *R. pentothenatexigens* JA575^T.

ANI comparison

With the availability of large numbers of genome sequences, in addition to ribosomal sequences and DNA–DNA hybridization, the ANI analysis became an additional measure to compare the similarity/relatedness of bacteria. In practice, bacteria with ANI >97% subjectively have only a few nucleotide differences and virtually no amino acid substitutions or insertions or deletions of either genes or amino acid residues. There would be no argument as to whether they were members of the same species. Bacteria with ANI values of 95–97 % will have minimal amino acid substitutions and virtually no insertions or deletions and would also be recognized as same species [12]. Bacteria with ANI values <90% will have insertions and deletions of whole genes, they will have substantial base changes, and most proteins will have at least a few amino acid substitutions plus a few amino acid insertions/deletions. In other words, they would be recognized in most cases as separate species. However, ANI values between 90 and 95% may be argued either way, and their assignment to a species could be difficult.

The genome sequence of *R. palustris* DSM 123^T [18, 19] is nearly identical to that of strain BisB5 as shown by an ANI value of 98.2%. According to the borderline of 95% proposed by Richter *et al.* [12] they represent strains of the same species and should both be recognized as proper strains of *R. palustris* as defined by van Niel and Pfennig and Trüper [1, 2]. With ANI values well below 90% they are clearly distinct at the species level from strains AAP120, HA2 and ATH 2.1.18 (See Table 1).

The genome sequence of *R. rutila* R1^T [20] is most closely related in ANI to strains CGA009, ATH 2.1.37, Eli 1980, DSM 126 and TIE1 (with ANI >97%, Table 1), indicating that they are likely to be the same species, but clearly distinct from *R. palustris* DSM 123^T and BisB5 (ANI <82%). An ANI value in the vicinity of 80% quite obviously separates these strains at the species level, i.e. as strains of *R. palustris* and *R. rutila*, respectively. Strains YSC3 and PS3 form a distinct cluster (ANI 95.8% to each other) with close association to (or identity with) *R. rutila* R1^T (see Table 1).

R. pentothenatexigens JA575^T has an ANI value of 88.6% to *R rutila* R1^T and 81.2% to *R. palustris* DSM 123^T, which indicates clearly the separation at species level. The genome sequence of *R. thermotolerans* JA576^T ([21]; accession number QUMP) had an ANI value of 100% to *R. pentothenatexigens* JA575^T (accession number QRDT). As an ANI value of 100% between two different strains is quite unlikely and *R. pentothenatexigens* and *R. thermotolerans* show clearly different phenotypic properties [8], this may be taken as an indication that the strains were inadvertently mixed up prior to genome sequencing. This discrepancy has to be resolved before the taxonomic status of the two species can be confirmed.

The genome sequence of *R. faecalis* JCM 11668^T [21] is also different from those of *R. palustris* DSM 123^T, *R. rutila* R1^T and *R. pentothenatexigens* (ANI 77.8–78.3%). Strain MAG1 is 97.7% identical to *R. faecalis* and obviously the same species. Strains PSBS and JSC-3b are closely related (ANI 93.7–95.2%) to *R. faecalis* JCM 11668^T and currently may be regarded as belonging to this species. In addition, strains DX1 and 420L belong to a single unnamed species (98% identical), but all other strains with genome sequences deposited in GenBank (XCP, AAP120, ATH 2.1.18, HaA2, B29, BisA53, BisB18, BAL398) are distinct from these, as well as from each other. As a matter of fact, according to ANI data, more detailed studies might prove that many more species of the genus *Rhodopseudomonas* are in culture than anticipated from the so far described species.

Table 1. Average nucleotide identity values of strains assigned to the genus *Rhodopseudomonas* arranged in order of relatedness

Strains highlighted in yellow are considered to belong to the same species, whereas those in green are likely to be closely related strains

ELI1980																									
99.8	CGA009																								
99.8	**99.9**	ATH 2.1.37																							
98.8	**99**	**99**	DSM126																						
97.4	**97.5**	**97.6**	**97.6**	TIE-1																					
97.2	**97.4**	**97.3**	**97.3**	**97.3**	*Rps. rutila* R1^{T}																				
92.0	**92.2**	**92.2**	**92.2**	**92.2**	**92.4**	PS3																			
92.1	**92.2**	**92.2**	**92.2**	**92.2**	**92.4**	**95.8**	YSC3																		
88	88.4	88.2	88.3	88.2	88.3	88.6	88.3	*Rps. pantothenatexigens* JA575^{T}																	
88.1	88.3	88.2	88.3	88.2	88.2	88.4	88.3	**100**	*Rps. thermotolerans* JA576^{T}																
88.1	88.2	88.2	88.4	88.3	88.4	88.6	88.7	89.6	89.6	DX1															
88.3	88.5	88.4	88.4	88.4	88.5	88.7	88.8	89.7	89.7	**98.0**	42OL														
87.9	88.1	88	88.1	88	88.1	88.2	88.3	89.3	89.3	88	88.3	XCP													
81.8	81.9	82	82	82.8	82.1	82.2	82.3	82.3	82.3	82.1	82.2	82.1	AAP120												
81.5	81.6	81.6	81.6	81.5	81.6	81.8	81.7	82.1	82.1	81.8	81.5	81.6	**87.1**	ATH 2.1.18											
81.6	81.8	81.7	81.8	81.7	81.8	81.9	81.9	82.3	82.4	82.3	82.4	81.8	**84.0**	**83.9**	HaA2										
81	81.1	81.1	81.2	81.1	81.2	81.3	81.4	81.6	81.6	81.3	81.6	81.2	83.3	82.9	**85.6**	*Rps. palustris*DSM123^{T}									
81.3	81.3	81.3	81.4	81.6	81.4	81.5	81.5	81.8	81.8	81.5	81.6	81.1	83.6	83	**85.7**	**98.2**	BisB5								
80.8	81	81	81	81	81.1	81.1	81.2	81.5	81.5	81.1	81.3	81.3	81.9	81.6	81.1	80.5	80.4	B29							
78.2	78.2	78.2	78.4	78.2	78.4	78.4	78.4	78.7	78.7	78.6	78.7	78.2	78.5	78.5	79.2	79.2	79.4	77.9	BisA53						
77.7	77.8	77.8	77.9	77.8	77.8	77.9	78	78.3	78.2	78	78	77.7	77.8	77.8	78.3	77.9	78.2	77.5	80.9	*Rps. faecalis* JCM11668^{T}					
77.7	77.7	77.8	77.8	77.8	77.8	77.8	77.8	78.2	78.2	77.9	77.9	77.7	77.8	77.8	78.3	78.1	78.2	77.4	81	**97.7**	MAG1				
77.7	77.9	77.8	77.8	77.8	77.8	77.8	77.9	78	78	77.9	77.9	77.7	77.8	77.8	78.3	78.1	78.2	77.5	81.1	**94.0**	**93.9**	PSBS			
77.8	77.9	77.9	77.8	77.7	77.9	77.9	77.8	78.1	78.1	78	78	77.6	77.8	77.8	78.3	78.1	78.7	77.5	81	**93.7**	**93.5**	**95.2**	JSC-3b		
78.3	78.3	78.3	78.2	78.1	78.2	78.3	78.2	78.5	78.5	78.4	78.5	77.9	78.3	78.4	79.2	79	79.2	77.9	81.4	79.3	79.3	79.3	79.3	BisB18	
78.6	78.7	78.7	78.6	78.5	78.6	78.7	78.6	79	79	78.7	78.8	78.7	79.1	78.8	80	79.4	79.7	78.2	79.5	77.8	78	77.9	77.9	80.5 BAL398	

Table 2. Amino acid sequences of cytochromes C2

(1) *R. rutila* R1^T and strain ATH 2.1.37, which are identical to one another and to those from the translated genome sequences. (2) Amino acid sequence of *R. palustris* ATH 2.1.6^T–KCM, which is identical to that from the translated genome sequence of strain DSM 123, but different from those in sequence 1 at 13 positions (yellow highlight)

1	QDAKAGEAVFKQCMTCHRADKNMVGPALGGVVGRKAGTAAGFTYSPLNHNSGEA GLVWTADNIINYLNDPNAFLKKFLTDKGKADQAVGVTKMTFKLANEQQRKDVVAYLATLK
2	QDAAKGEAVFKQCMTCHRADKNMVGPALGGVVGRKAGTAAGFTYSPLNHNSGEAGLVWTQ ENIIAYLPDPNAYLKKFLTDKGQADKATGSTKMTFKLANDQQRKDVAAYLATLK

Cytochrome c sequences

Strains from the van Niel collection were grown by one of us (T.E.M.) and the cytochrome composition was studied. The amino acid sequences of the cytochromes C2 and C556 from *R. palustris* strain ATH 2.1.6^T–KCM grown in our lab are in complete agreement with the translated genome sequences of *R. palustris* DSM 123^T and strain BisB5 [22, 23] (see Table 2).

In addition, the cytochrome amino acid sequences C2, C′ and C556 of *R. rutila* strains R1^T (strain obtained from T. Akiba) and ATH 2.1.37 (strain obtained from van Niel) were found to be identical to one another [22, 23] and to those translated from the genome sequences of R1^T, ATH 2.1.37, CGA9, TIE-1 and Eli1980. This fits very well into the cluster formed according to the ANI. The cytochromes C2 of *R. rutila* R1^T and of *R. palustris* ATH 2.1.6^T–KCM have been shown to differ in 13 amino acid positions ([22, 23] andTable 2).

Phylogenetic tree

The results of whole genome-based phylogenetic analysis of the *Rhodopseudomonas* genomes using RAxML [15, 16] are consistent with the ANI results presented above. The phylogenetic tree was generated using whole genome comparisons, with 872 aligned proteins from single-copy genes using RAxML within PATRIC [13]. As can be seen in Fig. 1, the *R. rutia* R1^T genome is clearly placed within a group of six strains (TIE-1, DSM 126, ATH 2.1.37, CGA009 and ELI 1980), and closely related to strains PS3 and YSC3, which based on ANI results all belong to the same species (red coloured in Fig. 1). As expected from the ANI results, *R. faecalis* JCM 11668^T and strain MAG1 form a distinct branch on the phylogenetic tree, closely related to strains JSC-3b and PSBS (green coloured in Fig. 1). The type strain of *R. palustris* DSM 123^T is located on a branch including strain BisB5 (blue coloured in Fig. 1), and somewhat more

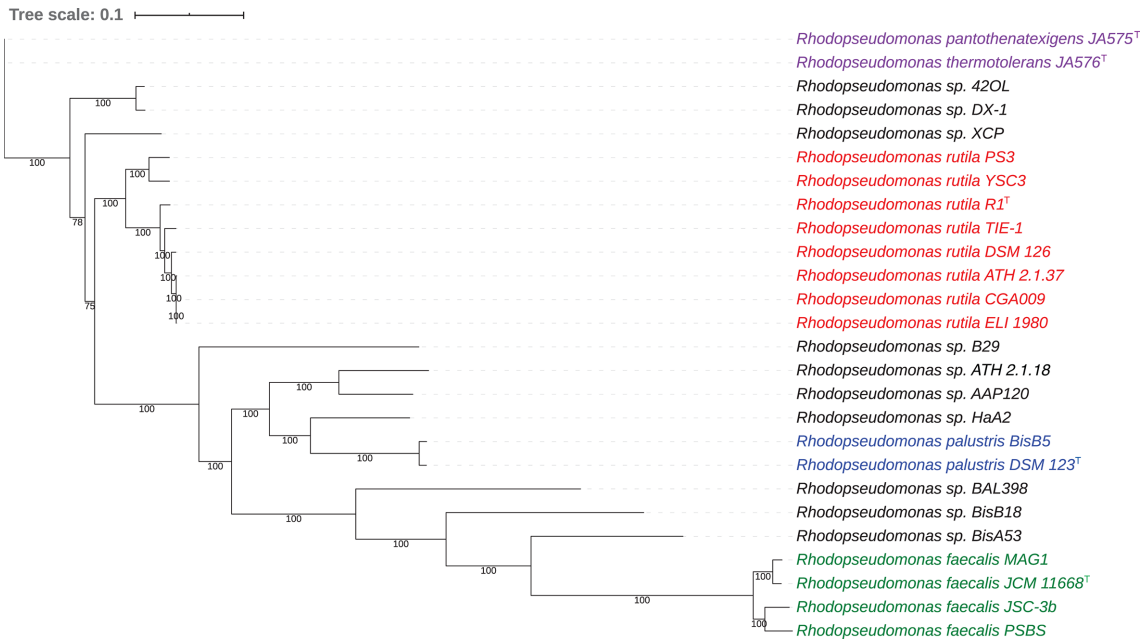

Fig. 1. Phylogenetic tree of *Rhodopseudomonas* strains based on closest relatives whole genome comparison. The phylogenetic tree was generated using the Codon tree method, which used PGFams as homology groups and analysed 872 aligned proteins (and coding DNA) from single-copy genes using RAxML [15, 16]. iTOL was used for the tree visualization [17]. Strains are coloured by group based on ANI calculations described in the text.

distant from strains HaA2, AAP120 and ATH 2.1.18, consistent with our ANI comparisons. The sequences of *R. pentothenatexigens* JA575 and *R. thermotolerans* JA576 are only distantly related to the other strains and identical based on the RAxML comparison (purple coloured in Fig. 1).

CONCLUSIONS

In order to solve the taxonomic confusion and correct the mistakes in regard to valid descriptions of *R. pseudopalustris* and *R. rutila*, we conclude the following on the basis of the available information. The problems started with the recognition of *R. rutila* as a heterotypic synonym of *R. palustris* by Hiraishi *et al.* [10] with reference to ATCC 17001, which had lost properties of the authentic original deposit. The problems continued with further studies that did not include the type strain of *R. rutila* R1^T nor the authentic type strains of *R. palustris* DSM 123^T and NBRC 100419^T (demonstrated here to be identical to the van Niel strain ATH 2.1.6) but instead referred to strain ATCC 17001 (demonstrated to be clearly different from the authentic type strain references ([11], this work).

In order to solve the question of the authenticity of the type strains of *R. palustris* in the different culture collections, we have included reference to cytochrome sequences from the strain obtained from its original source, the van Niel culture collection. This strain, referred to as ATH 2.1.6^T–KCM in this communication, was compared with the reference strain from DSMZ (DSM 123^T) and with the type strain of *R. rutila* R1^T which previously was shown to be identical on a species level to strains ATCC 17001 and CGA009 [10, 11]. As we found complete identity in cytochrome C2 sequences between the van Niel strain ATH 2.1.6^T–KCM and strain DSM 123^T, we conclude on the authenticity of the deposit at DSMZ as the authentic type of *R. palustris*. As also identity of the DSM 123^T deposit with NBRC 100419^T was confirmed earlier [11], we conclude that also the NBRC 100419^T represents the authentic type of *R. palustris*. Based on the phylogenetic tree, cytochrome C2 sequences and ANI values for strains ATH 2.1.6^T–KCM and BisB5, both should be regarded as strains of *R. palustris*.

Also, the authentic type of *R. palustris* DSM 123^T was assigned as type to another new species, *R. pseudopalustris*. This is of course obsolete and as a consequence of the incorrectly assigned type to *R. pseudopalustris*, the description of this species shall be regarded as illegitimate. *R. palustris* is the older species and DSM 123^T was first assigned as type to this species, which has to be considered with priority. All information collected and referred to *R. pseudopalustris* should be regarded as information belonging to *R. palustris*.

On the other hand the species name of *R. rutila* with its type R1^T was validly published. The transfer to *R. palustris* was premature because it was based on wrong assumptions and on the reference to a non-authentic type strain with properties as described for *R. rutila* Hiraishi *et al.* [10]. In fact, a number of strains shown to be closely related to strain R1^T ([10, 11, this study) should be considered strains of *R. rutila* and their properties regarded as properties of *R. rutila*, including ATCC 17001, ELI 1980, CGA009, TIE-1 and ATH 2.1.37.

In addition, strain ATCC 17001 has been shown by DNA–DNA hybridization analysis to be identical at the species level to *R. rutila* R1^T [10]. In consequence, and due to the fact that its properties no longer conform to the authentic type strain of *R. palustris* ATH 2.1.6^T–KCM=DSM 123^T=NBRC 100419^T, strain ATCC 17001 can no longer be regarded as a type strain of *R. palustris* but is a strain of *R. rutila*. It does not conform to the original or emended descriptions of the type strain of *R. palustris*.

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

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