Prescottia equi gen. nov., comb. nov.: a new home for an old pathogen

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Abstract:

The taxonomic status of *Rhodococcus equi*, originally isolated from foal specimens, has been the subject of discussion for a number of years. The chequered history of the taxon has prompted this polyphasic analysis of *R. equi* strains, close members of the genus *Rhodococcus* and representatives of other genera classified in the order *Corynebacteriales*, to establish the taxonomic position of this taxon. Thirty one *R. equi* strains, including the type strain, were examined for genotypic and numerical taxonomic properties. The resultant data are consistent with their classification in the order *Corynebacteriales,* but the *R. equi* strains formed a distinct phyletic clade away from representatives of other members of the genus *Rhodococcus* in the 16S rRNA gene tree. Representatives of this clade shared their highest pairwise 16S rRNA gene sequence similarities with the type strain of *Rhodococcus kunmingensis* (95.2 to 98.1%). However, the *R. equi* taxon was readily distinguished from *R. kunmingensis* and from the other members of the order *Corynebacteriales* using a combination of genotypic, chemotypic and phenotypic properties. On the basis of these data the *R. equi* strains are considered to represent a new genus. The name proposed for this taxon is *Prescottia gen. nov.,* with *Prescottia equi comb. nov.* as the type species containing the type strain, C 7^{T} (=ATCC 25729^{T}=ATCC 6939^{T} =CCUG 892^{T}=CIP 54.72^{T}=DSM 20307^{T}=HAMBI 2061^{T}=NBRC 14956^{T}=JCM 1311^{T}=JCM 3209^{T}=LMG 18452^{T}=NBRC 101255^{T} =NCTC 1621^{T}=NRRL B-16538^{T}=VKM Ac-953^{T}).

Introduction

In 1923, Magnusson isolated a strain of the primary causal agent of equine pneumonia and classified it in the genus *Corynebacterium* as *Corynebacterium equi* (Magnusson 1923). Subsequently, following a somewhat turbulent taxonomic history (Barton and Hughes 1980; Goodfellow and Jones 2012), a study by Goodfellow & Alderson (Goodfellow and Alderson 1977) led to the species being transferred to the genus *Rhodococcus* as *Rhodococcus equi*. The organism, a facultative intracellular parasite of macrophages, is an important pathogen of foals; it causes fatal lymphadenitis and ulcerative enteritis in 3 to 5 month old foals (Meijer and Prescott 2004; Giguere et al. 2011a; Giguere et al. 2011b; Prescott 1991).

More recently, *R. equi* has been recognised as an opportunistic pathogen of humans, especially immunocompromised patients; necrotizing pneumonia is the most common disease manifestation although the organism also causes extra-pulmonary infections (Kedlaya et al. 2001; Yamshchikov et al. 2010; Takai et al. 1994; Prescott 1991). Misidentification of *R. equi* strains in animals and humans as mycobacterial infections (Meijer and Prescott 2004) delays correct treatment of patients thereby causing relapses and fatalities, with mortality rates between 50-55% in patients with HIV, 20-25% in those with non-HIV compromised immunity and 11% in immunocompetent patients (Kedlaya et al. 2001). The importance of *R. equi* as a multihost pathogen has led to extensive studies of its virulence (von Bargen and Haas 2009; Giguere et al. 2011a) and to sequencing of the whole genome of a pathogenic strain (Letek et al. 2010).

The genus *Rhodococcus* currently encompasses 33 species with validly published names. Previous studies have shown that members of the genus can be assigned to three 16S rRNA gene clades which fall within the evolutionary radiation of the order *Corynebacteriales* (Jones and Goodfellow. 2012; Goodfellow and Jones 2012), namely the *R. equi*, *Rhodococcus erythropolis* and *Rhodococcus rhodochrous* clades (Goodfellow et al. 1998; McMinn et al. 2000; Jones et al. 2004; Jones and Goodfellow. 2012); the taxonomic integrity of these taxa are supported by specific 16S rRNA gene sequences (Goodfellow et al. 1998; Gurtler et al. 2004). The aim of the present polyphasic taxonomic study was to establish whether strains falling within the *R. equi* clade merit generic status.

Materials and methods

Thirty one *R. equi* strains, 15 strains representing other *Rhodococcus* species, along with representatives from the related mycolic acid containing genera *Corynebacterium*, *Dietzia*, *Gordonia*, *Mycobacterium*, *Nocardia*, *Tsukamurella* and *Williamsia*, including 59 type strains, were obtained from either private or public culture collections (Table S1). The organisms were maintained on glucose-yeast extract agar (GYEA; Gordon and Mihm 1962) at room temperature and as glycerol suspensions (20%, v/v) at -20°C. Fifteen strains were randomly selected as duplicates to establish test error in the numerical taxonomic and molecular fingerprinting studies.

Extraction of chromosomal DNA from each of the 129 isolates was carried out using the method of Kim et al. (1998). The strains were the subject of a composite DNA fingerprinting analysis based on two different repetitive DNA elements (rep-PCR; Versalovic et al. 1994) using BOX A1R (Pathom-aree et al. 2006) and ERIC (Versalovic et al. 1991) primer sets, and three individual amplified 16S ribosomal DNA restriction studies (ARDRA; Vaneechoutte et al. 1995) that used the restriction enzymes *Alu* I, *Hpa* II and *Cfr* I31.

The *rep*-PCR amplifications were performed in a 25 µl reaction mixture containing 1 µl template DNA (100ng), 1 x PCR buffer, 4 µM of primer BOXA1R (Pathom-aree et al. 2006) with 2 µM of primers ERIC 1R and ERIC 2 (Versalovic et al. 1991), 100 mM DMSO, 6 mM $MgCl_2$, 0.1 U Bio*Taq* DNA polymerase and 1.24 mM of each of the four dNTPs. Amplification with the primers was carried under the following conditions: initial denaturation step at 95°C for 5 minutes, 30 cycles of 95°C for 1 minute, 52°C for 1 minute and 65°C for 8 minutes with a final incubation at 65°C for 18 minutes. PCR amplifications of 16S rRNA genes were carried out according to the method of Kim et al. (Kim et al. 1998), with each PCR product digested singly for 2 hours at 37°C in final reaction volumes of 10 µl, consisting of 8.5 µl of PCR product, 1 µl of 1 x buffer Y^+/Tango™ and 0.5 U of restriction enzyme.

Cluster analysis of the *rep*-PCR fingerprints was based on band intensity using the unweighted-pair-group method with averages algorithm (UPGMA; Sokal and Michener 1958) and the Pearson's product-movement correlation coefficient (Pearson 1926). Each primer type was analysed separately. The similarity of the ARDRA band patterns generated by the digest with each restriction endonuclease were analysed separately using the UPGMA algorithm (Sokal and Michener 1958) and the Jaccard coefficient (Jaccard 1908). The *rep*-PCR and ARDRA fingerprint data were combined to give a consensus matrix.

The 129 strains and the 15 control duplicated cultures were examined for 96 unit characters (Table 2) using methods known to yield data of value for the classification and identification of mycolic acid-containing actinomycetes (Goodfellow and Alderson 1977; Goodfellow et al. 1998; Goodfellow et al. 1982a; Goodfellow et al. 1982b; Jones et al. 2008; Goodfellow and Jones 2012). Tolerance to antibiotics was

determined using antibiotic discs (Table 2; Oxoid Ltd., Wade Road, Basingstoke, UK) impregnated with specific concentrations of antibiotic in accordance with the British Society of Antimicrobial Chemotherapy (BSAC) guidelines (Andrews 2001). When zone sizes were equal to or greater than those specified by the BSAC guidelines, a negative (sensitive) result was scored; zone sizes measuring less than those specified by the guidelines were scored positive (resistant).

The test strains were also examined for their capacity to cleave a range of fluorogenic and chromogenic substrates (Table 2). The substrates were dissolved in double strength phosphate buffer to give a final concentration of 1 mM. Suspensions of two day old biomass cultured on GYEA were suspended to give a McFarland density of 3. Equal volumes of each suspension and substrate solution were dispensed into microtitre wells. The plates containing the fluorogenic substrates were read for fluorescence at excitation 365 nm, emission 440 nm and sensitivity 28; the chromogenic substrates were examined for absorbance at the wavelength of 405 nm. The resultant readings were recorded as time zero and after two days incubation at 30°C, the microtitre plates were read again at the same settings. Results of the tests were tabulated in the Microsoft Excel program (Microsoft Co., Seattle, USA) and transformed into two mutually exclusive states, scored positive (1) or negative (0), for the numerical taxonomic analysis. The tests were coded positive when the difference in fluorescent/absorbance intensities between the test and negative controls was more than 0 [$R_P = V_r - V_c - V_{a+b}$ (R_P, positive result; V_r, resultant reaction between test strain and conjugated substrate; V_c, value of cell inoculum alone; V_{a+b}, value of organism free control)].

Results

153
154 The results of the remaining phenotypic tests in the data matrix were converted to
155 binary format (1/0) written to a NTS file using Programmer's File Editor (PFE)
156 software. The final dataset was the subject of cluster analyses using the NTSYSpc
157 program (version 2.0; Numerical Taxonomy and Multivariate Analysis System; Rohlf
158 1998). Similarity values were calculated using the S_{SM} coefficient and clustering
159 accomplished with the UPGMA algorithm (Sneath and Sokal 1973); the results were
160 presented as a dendrogram. Co-phenetic correlation values (Sokal and Rohlf 1962)
161 were calculated using the NTSYS 'Coph' and 'Mxcomp' functions to estimate how
162 well the structure inherent in the similarity matrix was preserved by the clustering
163 procedure.
164
165 The phylogenetic positions of 26 *R. equi* isolates were determined in a 16S rRNA
166 gene sequence analysis. PCR amplification and direct sequencing of the purified
167 products were carried out using the method of Kim et al. (Kim et al. 1998) The almost
168 complete 16S rRNA gene sequences were aligned with corresponding sequences of
169 representatives of genera classified in the order *Corynebacteriales* (retrieved from the
170 DDBJ/EMBL/GenBank databases), using the CLUSTAL W alignment option and 16S
171 rRNA secondary structural information held in the MEGA 5 program (Tamura et al.
172 2011). Phylogenetic trees were inferred using the neighbor-joining (Saitou and Nei
173 1987), least squares (Fitch and Margoliash 1967), maximum-parsimony (Kluge and
174 Farris 1969) and maximum-likelihood (Felsenstein 1981) tree-making algorithms
175 from the MEGA 5 program (Tamura et al. 2011) and evolutionary distance matrices
176 prepared after Jukes and Cantor (1969). The resultant unrooted tree topologies were
177 evaluated in a bootstrap analysis (Felsenstein 1985) based on 1,000 resamplings.
178 Accession numbers for the sequences generated in this study are listed in Fig 1.
179
180 Heterogeneity within *Rhodococcus* was confirmed by comparison of 16S rRNA gene
181 sequences. Almost complete 16S rRNA gene sequences (1202 to 1518 nucleotides
182 [nt]) were generated for 26 of the *R. equi* strains. The phylogenetic relationships
183 between these organisms, the type strains of the other *Rhodococcus* species and
184 representatives of the 15 other genera classified in the order *Corynebacteriales* are
185 shown in Fig 1. The *R. equi* strains formed a distinct clade that was supported by all
186 four tree-making algorithms and by a bootstrap value of 88%, a result that is in good
187 agreement with corresponding data from previous studies (Goodfellow et al. 1998;

McMinn et al. 2000) and is underpinned by the study of Rainey et al. (Rainey et al. 1995), which also demonstrated the separation of *R. equi* from other members of the genus *Rhodococcus*. Nearly all of the remaining rhodococci were assigned either to the *R. erythropolis* or *R. rhodochrous* clades, a result in line with those from earlier studies (McMinn et al. 2000; Jones and Goodfellow. 2012; Goodfellow et al. 1998; Gurtler et al. 2004). The separation of the *R. equi* strains from these and related taxa is supported by specific nucleotide 16S rRNA gene signatures (Table 3). All of these results are consistent with the view that *R. equi* merits generic status (Jones and Goodfellow. 2012).

The 29 *R. equi* strains formed a distinct taxon based on the molecular fingerprint and numerical taxonomic data. The consensus dendrogram derived from the analyses of the *rep*-PCR and ARDRA fingerprint data (Fig 2) showed that the *R. equi* strains formed a distinct taxon that lay between corresponding groups encompassing members of the *R. erythropolis* and *R. rhodochrous* phyletic lines. All of these taxa were sharply separated from each other and from the representatives of mycolic acid-containing bacteria classified in the genera *Corynebacterium*, *Dietzia*, *Mycobacterium*, *Nocardia*, *Tsukamurella* and *Williamsia*. Similarly, the *R. equi* strains formed a distinct cluster defined at the 84.5 % similarity level in the complementary numerical taxonomic study (Fig 3). Once again, this taxon was distinct from members of the *R. erythropolis* and *R. rhodochrous* groups and from clusters composed of representatives of the other mycolic acid-containing genera. It is evident from both analyses that the genus *Rhodococcus* is a polyphyletic taxon, as highlighted in an earlier study (Rainey et al. 1995). Confidence can be placed in the present results as the duplicated *Rhodococcus* strains clustered together in the molecular fingerprint analysis while the test error recorded for these strains in the numerical taxonomic study was low (3.6%), a result well below the 10% cut off recommended previously (Sneath and Johnson 1972). The cophenetic correlation value obtained in the S_{SM}-UPGMA analysis was high at 0.72.

Discussion

Cumulatively, the chemotaxonomic, molecular systematic and numerical taxonomic data show that *R. equi* can be distinguished readily from other members of the genus

Rhodococcus and from the remaining genera classified in the order *Corynebacteriales* (Table 4). Consequently, we propose that *R. equi* be reclassified as a new genus, named *Prescottia* with *Prescottia equi* as the type species.

This change will still enable medical and veterinary clinical diagnosis to proceed with caution using the same diagnostic criteria as currently used for *R. equi* (Giguere and Prescott 1997; Prescott 1991). As *R. equi* is the only documented *Rhodococcus* species to contain the virulence plasmids which encode the VapA protein (in foal and some human isolates) or the homologous VapB protein (in porcine and human isolates) (Giguere et al. 2011a; Tkachuk-Saad and Prescott 1991; Giguere et al. 2011b), PCR amplification of the *vapA* gene for equine isolates (Giguere et al. 2011b) and/or of the *vapB* gene for isolates from human clinical sources are thus of major diagnostic importance. Moreover, the numerical taxonomic analysis revealed phenotypic characteristics of diagnostic potential for the recognition of *R. equi* strains (Table 5). All 31 *R. equi* strains, including the type strain, were positive in the forty two enzyme tests listed in Table 5 with the exception of strain N1310 which was unable to hydrolyse L-arginine-7AMC, L-glycine-7AMC-hydrogen bromide or L-ornithine-7AMC-dihydrochloride. Integration of the tests summarised in Table 5 into diagnostic keys should facilitate the improved recognition of this pathogen.

The degree of confidence that can be placed in a classification is reflected in the congruence found when the same strains are assigned to corresponding taxa based on different, but complementary, taxonomic criteria. It is, therefore, encouraging that in the present study that the *R. equi* strains were assigned to taxa distinct from other rhodococci based on both the molecular fingerprint and numerical taxonomic data (Figs 2 and 3). Excellent congruence was found between the composition of the two *R. equi* subgroups recovered in these analyses though an exception was *R. equi* N1310, which was recovered in subgroup 1 in the former analysis and in subcluster 2 in the latter one. However, in each analysis the type strain of *R. equi* and 15 related strains, including isolates from animal, human and environmental sources (Table S1), formed a homogeneous taxon, the taxonomic status of which is underpinned by pyrolysis mass spectrometric and previous numerical phenetic data (Goodfellow and Alderson 1977; Goodfellow et al. 1982a; Goodfellow et al. 1982b; McMinn et al. 2000).

It can be concluded from the genotypic and phenotypic data that the taxon containing isolate C 7^T corresponds to *R. equi* (Goodfellow et al. 1998; Magnusson 1923). It is, therefore, proposed that this species be recognised as the type species of the new genus *Prescottia*. However, additional work needs to be carried out to establish whether the subgroup 2 strains should be recognised as a second *Prescottia* species. Further work is also required to clarify the taxonomic status of *R. kunmingensis* DSM 45001^T which lies towards the periphery of the *Prescottia* 16S rRNA gene clade (Fig. 1). The type strains of *R. equi* and *R. kunmingensis* share a DNA:DNA relatedness value of 34.4 ± 10% (Wang et al. 2008), a value well below the cut-off point recommended for the delineation of bacterial species (Wayne et al. 1987).

Description of *Prescottia* gen. nov.

Prescottia (Pres.cot'ti.a. fem. n. *Prescottia*) named after John Prescott to celebrate his many contributions towards unravelling the pathogenicity of *Rhodococcus equi*.

The description is based on data taken from this and previous studies (Collins et al. 1982a; Collins et al. 1982b; Collins et al. 1977; Collins et al. 1979; Collins et al. 1985; Cummins and Harris 1956; Goodfellow and Alderson 1977; Keddie and Cure 1977; Komura et al. 1975; Nishiuchi et al. 2000; Schleifer and Kandler 1972; Uchida and Aida 1977; Mordarski et al. 1980b; Mordarski et al. 1980a; Zakrzewska-Czerwinska et al. 1988) (Table 4).

Aerobic, Gram-positive, acid-alcohol fast, nonmotile, pleomorphic actinomycete which may show traces of elementary branching at early stages of growth. Whole-organism hydrolysates are rich in 2, 6-diaminopimelic acid, arabinose and galactose. The peptidoglycan is of the A1γ type. Muramic acid moieties are N-glycolated. Cells contain diphosphatidylglycerol, phophatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides as major polar lipids; complex mixtures of straight chain saturated, monounsaturated and branched chain fatty acids and dihydrogenated menaquinones with eight isoprene units as the predominant isoprenologue. Mycolic acids have 28 to 50 carbon atoms and up to four double bonds. The fatty acids released on pyrolysis gas chromatography of mycolic acid esters have 12 to 16 carbon atoms. The DNA G + C content ranges from 69-72 mol%. The genus *Prescottia*, as determined by 16S rDNA gene sequence analyses, is a member of the order *Corynebacteriales*.

Isolated from soil and intestinal tracts and faeces of animal species. Causes equine pneumonia in foals and is an opportunistic pathogen of humans.

Description of *Prescottia equi* comb nov.

Prescottia equi (e'qui. L. n. *equus* horse; L. gen. n. of the horse).

In addition to the characteristics given in the genus description, the species has the following properties based on the results of this and previous studies (Barton and Hughes 1980; Goodfellow and Alderson 1977; Goodfellow et al. 1982a; Mordarski et al. 1980b; Mordarski et al. 1980a).

In smears cells often show a clumping or palisade arrangement or L- or V- shaped elements. Smears from liquid cultures sometimes show branching filamentous forms with swollen ends. Smooth, shiny, pale pink colonies with entire margins are produced on glucose-yeast extract agar; abundant slime which may drop onto the lids of inverted Petri dishes may be produced during incubation. Grows from 5 to 40^{o}C, optimally around 30^{o}C. Phosphatase positive but negative for arbutin and esculin hydrolysis. Reduction of nitrate to nitrite and hydrolysis of urea are variable. Degrades Tweens 20, 40, 60 and 80 but not arbutin, cellulose, chitin, guanine, hypoxantrine, L-tyrosine, uric acid or xanthine. Degradation of adenine is variable. Cleaves the following exopeptides: D-alanine-7-AMC trifluoroacetate, L-arginine-7-AMC, L-glutamate 7-AMC, L-glycine 7-AMC- hydrogen bromide, L-histidiine 7-AMC, L-*iso*-leucine 7-AMC-trifluoroactetate, L-leucine-7-AMC, L-lysine 7-AMC-acetate, L-methionine 7-AMC-acetate, L-ornithine-7-AMC dihydrochloride, L-phenylalanine 7-AMC-trifluoroacetate, L-proline-7-AMC hydrogen bromide, L-threonine 7-AMC, L-tyrosine 7-AMC and N-benzyloxycarbonyl glycyl-prolyl-7AMC. Cleaves the following glycosides: 4MU-αcetyl-β-D-glucosaminide, 4MU-β-D-fucoside, 4MU-β-D-galactoside, 4MU-α-D-glucoside, 4MU-β-D-glucoside, 4MU-β-D-glucuronide, 4MU-α-D-mannopyranoside, 4MU-β-D-ribofuranoside and 4MU-B-D-xylanopyranoside. Cleaves the following inorganic esters: dihydroumbelliferone, 4-methyl-7-nitrocoumarin, 4MU-phosphate disodium salt, organic esters, 4MU-acetate, 4MU-butyrate, 4MU-elaidate, 4MU-*p*-guanidinobenzoate, 4MU-heptanoate, 4MU-laurate, 4MU-nonanoate, 4MU-propionate and 4MU-stearate. In addition, the

chromogenic substrates *o*-nitrophenyl-myrisate, *p*-nitrophenyl-α-L-fucoside, *p*-nitrophenyl-phenylphosphonate, *p*-nitrophenyl-phosphorylcholine, *p*-nitrophenyl-α-L-rhannopyranoside and *p*-nitrophenol-α-L-xylopyranoside are cleaved.

Grows on adonitol, amygdalin, L-arabinose, cellobiose, erythritol, glycerol, glycogen, inositol, inulin, maltose, mannitol, α- and β-methyl-D-glucoside, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose and xylose as sole carbon sources but not on xylitol. Sensitive to (μg ml): cephalexin (30), clindamycin (2), colistin sulphate (25), cotrioxide (25), fusidic acid (10), nalidixic acid (30), novobiocin (5) and penicillin G, but is resistant to erythromycin (4), gentamicin (8), lincomycin (64), minocycline (0.125), neomycin (8), novobiocin (4), streptomycin (4) and tobramycin (8).

The DNA G + C content of the type strain is 70.4 mol%.

The type strain, C 7^T (= ATCC 25729^T = ATCC 6939^T = CCUG 892^T = CIP 54.72^T = DSM 20307^T = HAMBI 2061^T = NBRC 14956^T = JCM 1311^T = JCM 3209^T = LMG 18452^T = NBRC 101255^T = NCTC 1621^T = NRRL B-16538^T = VKM Ac-953^T) was isolated from a lung abscess of a foal.

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558 Table 2 Unit characters scored in the phenotypic characterisation of the test strains

Unit character	Unit character
A. Biochemical tests (%, w/v):	**D. Enzyme tests**
Aesculin (0.1)*,[2]	**Cleavage of 7-amino-4-methylcoumarin substrates (7AMC):**
Arbutin (0.1)*,[2]	Exopeptidases:
Nitrate reduction (0.1)	D-alanine-7AMC-trifluoroacetate[3]
Nitrite reduction (0.1)	L-arginine-7AMC[3]
Urease production (2.0)[2]	L-asparagine-7AMC-trifluoroacetate[3]
B. Degradation tests (%, w/v):	L-aspartate-7AMC[3]
Adenine (0.4)*,[2]	L-glutamate-7AMC[3]
Casein (0.1)*,[2]	L-glycine-7AMC-hydrogen bromide[3]
DNA (0.3)*,[2]	L-histidine-7AMC[3]
Guanine (0.4)*,[2]	*iso*-L-leucine-7AMC-trifluoroacetate[3]
Hypoxanthine (0.4)*,[2]	L-leucine-7AMC[3]
RNA (0.3)*,[2]	L-lysine-7AMC-acetate[3]
Starch (0.1)*,[2]	L-methionine-7AMC-acetate[3]
Tributyrin (1.0 %)*,[2]	L-ornithine-7AMC-dihydrochloride[3]
L-Tyrosine (0.5)*,[2]	L-phenylalanine-7AMC-trifluoroacetate[3]
Tween 20 (1.0 %)*,[2]	L-proline-7AMC-hydrogen bromide[3]
Tween 40 (1.0 %)*,[2]	L-pyroglutamate-7AMC[3]
Tween 60 (1.0 %)*,[2]	L-threonine-7AMC[3]
Tween 80 (1.0 %)*,[2]	L-tyrosine-7AMC[3]
Uric acid (0.5)*,[2]	N-benzyloxycarbonyl-arginine-7AMC-hydrochloride[3]
Xanthine (0.4)*,[2]	N-benzyloxycarbonyl-glycyl-prolyl-7AMC[3]
C. Morphological tests	**Cleavage of 4-methylumbelliferone substrates (4MU):**
Growth on glucose-yeast extract agar:	Glycosides:
Light orange*	4MU-N-acetyl-ß-D-glucosaminide[3]
Light yellow pink*	4MU-α-L-arabinopyranoside[3]
Pale orange yellow*	4MU-ß-D-cellobioside[3]
Slight yellow pink*	4MU-ß-D-fucoside[3]
Very orange*	4MU-α-D-galactopyranoside[3]
Very red*	4MU-ß-D-galactoside[3]
Very yellow*	4MU-α-D-glucoside[3]
Yellow white*	
Mucoid*	
Rough*	
Smooth*	

Unit character	Unit character
4MU-ß-D-glucoside[3]	*p*-np phenyl phosphonoate[3]
4MU-ß-D-glucuronide[3]	*p*-nitrophenyl phosphorylcholine[3]
4MU-α-D-mannopyranoside[3]	**E. Tolerance tests**
4MU-ß-D-mannopyranoside[3]	**Sensitivity to antibiotics (µg/ml):**
4MU-ß-D-ribofuranoside[3]	Aminocoumarin:
4MU-ß-D-xylopyranoside[3]	Novobiocin (5)[1]
Inorganic esters:	Cephalosporin:
8-acetyl-7-hydroxy-4-methylcoumarin[3]	Cephalexin (30)[1]
Dihydroumbelliferone[3]	Fusidane:
4-methyl-7-nitrocoumarin[3]	Fusidic acid (10)[1]
4MU-phosphate disodium salt[3]	Glycopeptides and peptide:
4MU-sulphate potassium salt[3]	Bacitracin (10)[1]
Organic esters:	Macrolide:
4MU-acetate[3]	Erythromycin (5)[1]
4MU-butyrate[3]	Penicillin:
4MU-elaidate[3]	Penicillin G (1)[1]
4MU-*p*-guanidinobenzoate[3]	Polymyxin:
4MU-heptanoate[3]	Colistin sulphate (25)[1]
4MU-laurate[3]	Quinolone:
4MU-nonanoate (genzyme)[3]	Ciprofloxacin (1)[1]
4MU-palmitate[3]	Nalidixic acid (30)[1]
4MU-proprionate[3]	Tetracycline:
4MU-stearate[3]	Tetracycline hydrochloride (10)[1]
Cleavage of nitrophenol (np):	Miscellaneous:
Glycosides:	Clindamycin (2)[1]
p-np-α-L-fucoside[3]	Cotrimaxazole (25)[1]
p-np-α-L-rhamnopyranoside[3]	
p-np-α-L-xylopyranoside[3]	
Inorganic esters:	
o-np myrisate[3]	

* Tests carried out using a multipoint inoculator (Goodfellow et al. 1992); Tests carried out using 0.5[1]; 2.5[2] and 3.0[3] McFarland inoculum densities.

568 **Table 3. Nucleotide signatures that separate *R. equi* from closely related**
569 ***Rhodococcus* clades defined in the 16S rRNA sequence analysis[a]**
570

Position[b]	*Nocardia* spp.	*R. corynebacterioides*	*R. equi*	*R. erythropolis*	*R. kunmingensis*	*R. rhodochrous*
40-42	G:S	G:Y	G:S	G:C	G:S	G:C
43	K	G	G	G	G	G
44	Y	Y	U	U	U	U
64-65	W:R	A:A	A:A	A:G	A:G	A:R
70	H	W	U	U	U	U
75	S	-	C	C	C	C
78	Y	-	-	-	-	A
84-88	T:C	C:C	Y:G	K:Y	C:C	Y:B
102	C	U	Y	C	C	Y
122	C	A:C	A:C	A:C	C:N	A:C
127-139	Y:Y	G:C	K:C	G:C	G:C	G:C
182-185	-	C:U	G:C	-	G:U	-
191-196	-	C:U	G:G	-	U:G	-
199	K	G	U	G	U	G
207	D	A	G	R	A	R
216	R	A	A	A	A	W
217-218	Y:Y	U:C	C:U	Y:Y	Y:Y	Y:Y
223-224	Y:R	C:A	C:A	C:A	C:A	C:A
292	R	G	G	A	G	G
303	Y	C	Y	U	C	Y
380-384	R:C	C:C	A:C	R:C	C:C	M:C
459	H	Y	A	N	C	M
490	U	C	C	C	U	B
579	U	C	C	C	C	C
610-615	-	C:A	U:G	-	U:G	C:A
623-627	-	-	C:A	-	-	-
634	R	A	G	A	G	A
760	A	G	G	G	G	G
835-835	-	-	U:C	-	-	-
998	C	C	U	Y	C	Y
100-1008	C:Y	C:C	C:C	Y:Y	C:C	C:Y
1017-1023	R:Y	C:C	C:C	B:Y	C:C	R:Y
1040	-	G	A	R	G	R
1119-1121	Y:R	C:G	C:G	U:A	U:A	Y:R
1132-1139	R:Y	R:Y	G:C	R:Y	G:C	R:B
1150-1152	Y:R	C:G	C:G	U:A	U:A	Y:R
1335	Y	C	C	C	C	C

[a]Degenerate nucleotide codes: R (A+G), Y (C+T), M (A+C), S (G+C), W (A+T), B (not A), N (any), K (G+T).
[b]Numbering based on *Escherchia coli*.

Table 4. Characteristics of wall chemotype IV genera classified in the order *Corynebacteriales*

Characters	Genera																
	Precottia	***Amycolicoccus***	***Corynebacterium***	***Dietzia***	***Gordonia***	***Hoyosella***	***Millisia***	***Mycobacterium***	***Nocardia***	***Rhodococcus***	***Segniliparus***	***Skermania***	***Smaragdicoccus***	***Tomitella***	***Tsukamurella***	***Turicella***	***Williamsia***
Cell morphology	Rod-cocus growth cycle, with trace elements of branching	Coccoid cells	Pleomorphic rods, often club-shaped; commonly in angular and palisade arrangement	Short rods and cocci	Rods and cocci or moderately branching hyphae	Coccoid cells occurring singly, in pairs, in tetrads or in small clumps	Characteristic rudimentary right angled branching	Rods, occasionally branched filaments which fragment into rods and coccoid elements	Mycelium which fragments into rods and cocci	Rods to extensive substrate mycelium; the latter fragments into irregular rods and cocci	Rods	Mycelium resembling a pine tree	Coccoid cells	Irregular rods that exhibit snapping division. coccoid rods apparent after prolonged culture	Rods occur singly, in pairs or in masses; coccobacillary forms occur	Single cell, arranged in V-forms or palisades	Thin irregular rods or cocci occur singly or in small clusters
Aerial hyphae	Absent	Absent	Absent	Absent	Absent	ND	Present	Usually absent	Present	Absent	Absent	Present but not visible to the naked eye	Absent	Absent	Absent	Absent	Present
Growth of visible colonies (days)	1-2	1-2	1-2	1-3	1-3	1-3	1-3	2-40	1-5	1-3	3-4	9-21	7	ND	1-3	1-2	1-4
Acid-fastness	Acid-alcohol-fast	ND	Sometimes weakly acid-fast	Not acid fast	Partially acid-alcohol-fast	weakly acid-fast	Acid-alcohol-fast	Strongly acid-fast	Partially acid fast	Partially acid-fast at some stage of the growth cycle	Acid-alcohol-fast	Not acid fast	ND	ND	Partially acid-alcohol-fast	ND	ND
Strictly aerobic	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Fatty acid composition[a]	S, U, T, T16[g]	S, U	S, U[b]	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U, T	S, U	S, U	S, U, T	S, U, T	S, U, T
Major menaquinone(s)[c]	MK-8(H_2)	MK-7, MK-8	MK-8(H_2)	MK-8(H_2)	MK-9(H_2)	MK-8	MK-8(H_2)	MK-9(H_2)	MK-8(H_4,ω-cycl)[f]	MK-8(H_2)	ND	MK-8(H_4,ω-cycl)	SQA-8(H_4 ω cycl) and SQB (H_4 dicycl)	MK-9(H_2)	MK-9	MK-10, MK-11	MK-9(H_2)
Muramic acid type	Glycolated	ND	Acetylated	Acetylated	Glycolated	Acetylated	Glycolated	Glycolated	Glycolated	Glycolated	ND	Glycolated	Glycolated	Glycolated	Glycolated	ND	Glycolated
Mycolic acid pattern[d]	Single spot	None	Single spot	Single spot	Single spot	ND	Single spot	Multiple spots	Single spot	Single spot	Multiple spots	Single spot	ND	ND	Two spots	None	Single spot
Mycolic acids:																	
Overall size (number of carbons)	28-50	ND	22-38	34-38	46-77	ND	44-52	60-90	46-64	30-54	ND	58-64	43-49	42-52	62-78	ND	50-56
Number of double bonds	0-4	ND	0-2	0-1	1-6	ND	ND	1-4	0-3	0-4	ND	2-6	ND	ND	1-7	ND	ND
Fatty acids released on pyrolysis	12-16	ND	8-18	ND	16-18	ND	ND	22-26	12-18	12-16	ND	16-20	ND	ND	22-26	ND	ND
Phosphatidylethanlamine present in polar lipid patterns	+	+	-[f]	+	+	+	+	+	+	+	ND	+	+	+	+	ND	+
DNA G + C content (mol%)	69-72[h]	60.04	51-67	66-73	63-69	49.3	64.7	57-73	63-72	63-73	68-72	67.5	63.7	69.3-71.6	68-78	65-72	64-65

Symbols: +, positive; -, negative; and ND, not determined. Data taken from Adachi *et al.* (Adachi et al. 2007), Butler *et al.* (Butler et al. 2005), Cummins *et al.* (Cummins and Harris 1956), Keddie *et al.* (Keddie and Cure 1977), Komura *et* al. (Komura et al. 1975), Schleifer *et al.* (Schleifer and Kandler 1972), Soddell *et al.* (Soddell et al. 2006) and Uchida *et al.* (Uchida and Aida 1977). [a], Abbreviations: S, straight chain; U, unsaturated; T, tuberculostearic acid (10-methyloctadecanoic acid). [b], *C. bovis*, *C. minutissimum*, *C. urealyticum* and *C. variabile* contain tuberculostearic acid (Collins et al. 1982a; Kämpfer et al. 1999; Lechevalier et al. 1977)[c], Examples of abbreviations: MK-9(H_2), menaquinone with two of the nine isoprene units hydrogenated (Collins et al. 1979; Collins et al. 1985; Collins et al. 1977); SQA and SQB, smaradiquinones A and B. [d], Number of mycolic acid spots produced from whole-organism methanolystaes (Minnikin et al. 1980; Minnikin et al. 1993; Minnikin et al. 1975; Barton et al. 1989; Collins et al. 1982a; Hsu et al. 2011; Nishiuchi et al. 2000)). e, In mycobacterial mycolic acids, double bonds may be converted to cyclopropane rings; methyl branches and other oxygen functions may be present. f, Present in *Corynebacterium bovis* and *C. urealyticum* (Kämpfer *et al*., (Kämpfer et al. 1999)). g, carbon 16 version of 10-methyloctadecanoic acid present in half of the *Prescottia* strains. h DNA G+C content of *P. equi* (Mordarski et al. 1980b; Mordarski et al. 1980a; Zakrzewska-Czerwinska et al. 1988)

Table 5. Key Phenotypic Characteristics of *R. equi* strains from subclusters 1 and 2.

Tests	Subcluster 1 and 2 N = 31
Biochemical:	
Arbutin	-*
Nitrite	-
Degradation:	
Casein	-
Guanine	-
Hypoxanthine	-
Tween 20	+
Tween 60	+
Tween 80	+
Xanthine	-
Enzyme tests:	
L-Arginine-7AMC	+*
L-Glutamate-7AMC	+
L-Glycine-7AMC-hydrogen bromide	+*
L-Histidine-7AMC	+
Iso-L-Leucine-7AMC-trifluoroacetate	+
L-Leucine-7AMC	+
L-Lysine-7AMC-acetate	+
L-Methionine-7AMC-acetate	+
L-Ornithine-7AMC-dihydrochloride	+*
L-Phenylalanine-7AMC-trifluoroacetate	+
L-Proline-7AMC-hydrogen bromide	+
L-Threonine-7AMC	+
L-Tyrosine-7AMC	+
Z-Glycyl-Prolyl-7AMC	+
4MU-N-acetyl-ß-D-glucosaminide	+
4MU-ß-D-fucoside	+
4MU-ß-D-galactoside	+
4MU-α-D-glucoside	+
4MU-ß-D-glucoside	+
4MU-ß-D-glucuronide	+
4MU-α-D-mannopyranoside	+
4MU-ß-D-ribofuranoside	+
4MU-ß-D-xylopyranoside	+
Dihydroumbelliferone	+
4-methyl-7-nitrocoumarin	+
4mu-phosphate disodium salt	+
4mu-acetate	+
4mu-butyrate	+
4mu-elaidate	+
4mu-p-guanidinobenzoate	+
4mu-heptanoate	+
4mu-laurate	+
4mu-nonanoate (genzyme)	+
4mu-proprionate	+
4mu-stearate	+
p-np-α-L-fucoside	+
p-np-α-L-rhamnopyranoside	+
p-np-α-L-xylopyranoside	+
o-np myrisate	+
p-np phenyl phosphonoate	+
p-nitrophenyl phosphorylcholine	+
Tolerance tests:	
Penicillin	+
Clindamycin	+

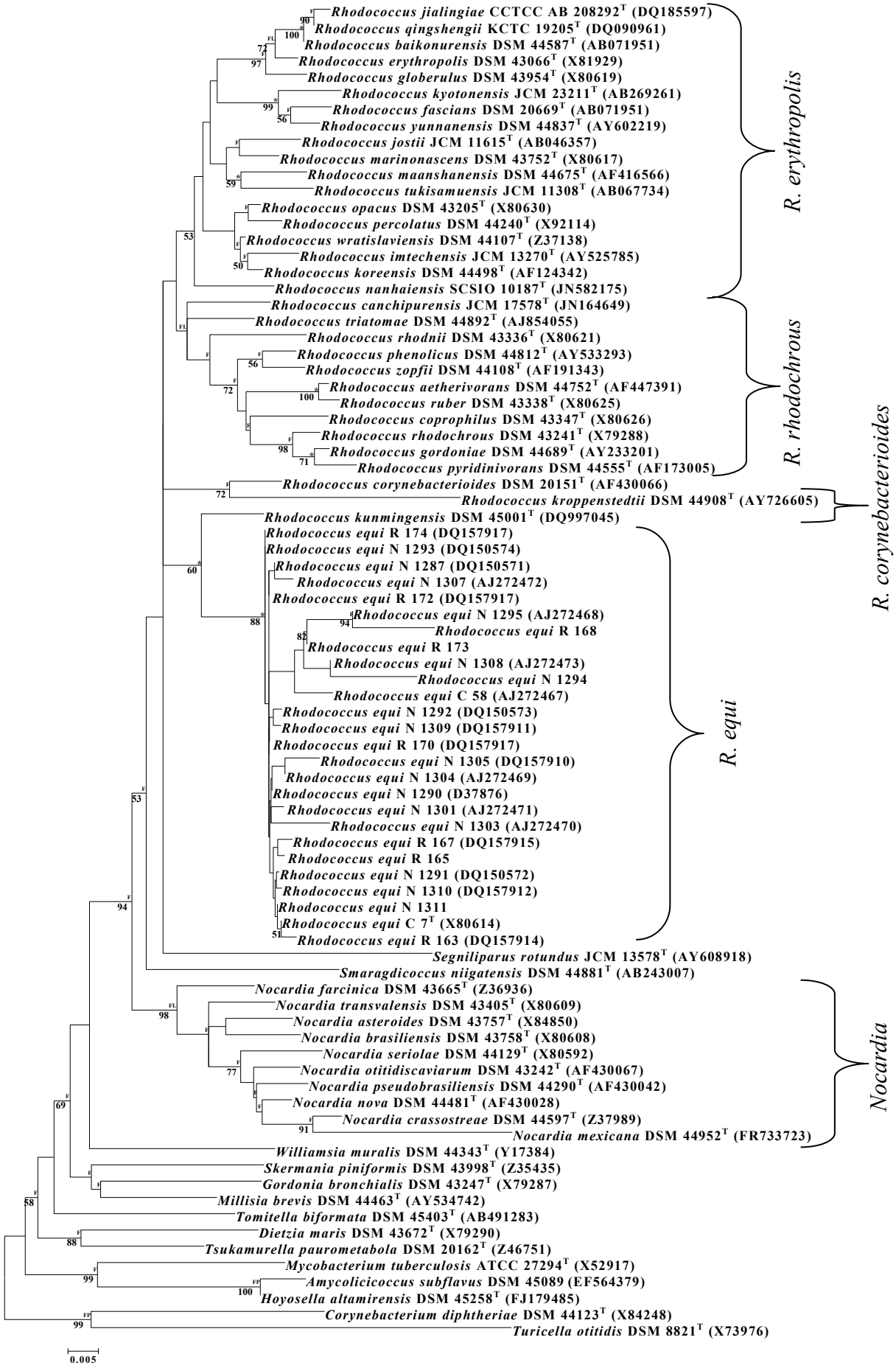
Symbols: +, positive; -, negative; *, a single strain from subcluster 2 (N1310) produced the opposite result to that recorded.

Legend for Figures

Fig. 1. Neighbor-joining tree (Saitou and Nei 1987) based on a nearly complete 16S rRNA gene sequences showing the position of the *R. equi* strains and representatives of genera classified in the order *Corynebacteriales*. Asterisks indicate branches of the tree that were also found with the least-squares (Fitch and Margoliash 1967) maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Kluge and Farris 1969) tree-making algorithms; the symbols F, L and P indicate branches recovered using the least-squares, maximum-likelihood and maximum-parsimony methods, respectively. The numbers at the nodes indicate levels of bootstrap support based on a neighbor-joining analysis of 1000 re-sampled datasets; only values above 50% are given. The scale bar indicates 10 substitutions per nucleotide position. ^{T}, type strain.

Fig. 2. A consensus dendrogram of the representatives of genera classified in the order *Corynebacteriales* based on the Pearson-UPGMA (Pearson 1926) and S_J-UPGMA (Jaccard 1908; Sokal and Michener 1958) analysis of the *rep*-PCR and ARDRA fingerprints showing relationships between the *R. equi* isolates and reference strains SMC, single-membered cluster.

Fig. 3. Dendrogram showing relationships between the *R. equi* isolates and the representatives of other mycolic acid containing taxa defined in the S_{SM}-UPGMA analysis.


Rhodococcus jialingiae CCTCC AB 208292T (DQ185597)
Rhodococcus qingshengii KCTC 19205T (DQ090961)
Rhodococcus baikonurensis DSM 44587T (AB071951)
Rhodococcus erythropolis DSM 43066T (X81929)
Rhodococcus globerulus DSM 43954T (X80619)
Rhodococcus kyotonensis JCM 23211T (AB269261)
Rhodococcus fascians DSM 20669T (AB071951)
Rhodococcus yunnanensis DSM 44837T (AY602219)
Rhodococcus jostii JCM 11615T (AB046357)
Rhodococcus marinonascens DSM 43752T (X80617)
Rhodococcus maanshanensis DSM 44675T (AF416566)
Rhodococcus tukisamuensis JCM 11308T (AB067734)
Rhodococcus opacus DSM 43205T (X80630)
Rhodococcus percolatus DSM 44240T (X92114)
Rhodococcus wratislaviensis DSM 44107T (Z37138)
Rhodococcus imtechensis JCM 13270T (AY525785)
Rhodococcus koreensis DSM 44498T (AF124342)
Rhodococcus nanhaiensis SCSIO 10187T (JN582175)
Rhodococcus canchipurensis JCM 17578T (JN164649)
Rhodococcus triatomae DSM 44892T (AJ854055)
Rhodococcus rhodnii DSM 43336T (X80621)
Rhodococcus phenolicus DSM 44812T (AY533293)
Rhodococcus zopfii DSM 44108T (AF191343)
Rhodococcus aetherivorans DSM 44752T (AF447391)
Rhodococcus ruber DSM 43338T (X80625)
Rhodococcus coprophilus DSM 43347T (X80626)
Rhodococcus rhodochrous DSM 43241T (X79288)
Rhodococcus gordoniae DSM 44689T (AY233201)
Rhodococcus pyridinivorans DSM 44555T (AF173005)
Rhodococcus corynebacterioides DSM 20151T (AF430066)
Rhodococcus kroppenstedtii DSM 44908T (AY726605)
Rhodococcus kunmingensis DSM 45001T (DQ997045)
Rhodococcus equi R 174 (DQ157917)
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Rhodococcus equi N 1287 (DQ150571)
Rhodococcus equi N 1307 (AJ272472)
Rhodococcus equi R 172 (DQ157917)
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Rhodococcus equi R 168
Rhodococcus equi R 173
Rhodococcus equi N 1308 (AJ272473)
Rhodococcus equi N 1294
Rhodococcus equi C 58 (AJ272467)
Rhodococcus equi N 1292 (DQ150573)
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Rhodococcus equi R 170 (DQ157917)
Rhodococcus equi N 1305 (DQ157910)
Rhodococcus equi N 1304 (AJ272469)
Rhodococcus equi N 1290 (D37876)
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Rhodococcus equi N 1303 (AJ272470)
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Rhodococcus equi R 165
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Rhodococcus equi N 1310 (DQ157912)
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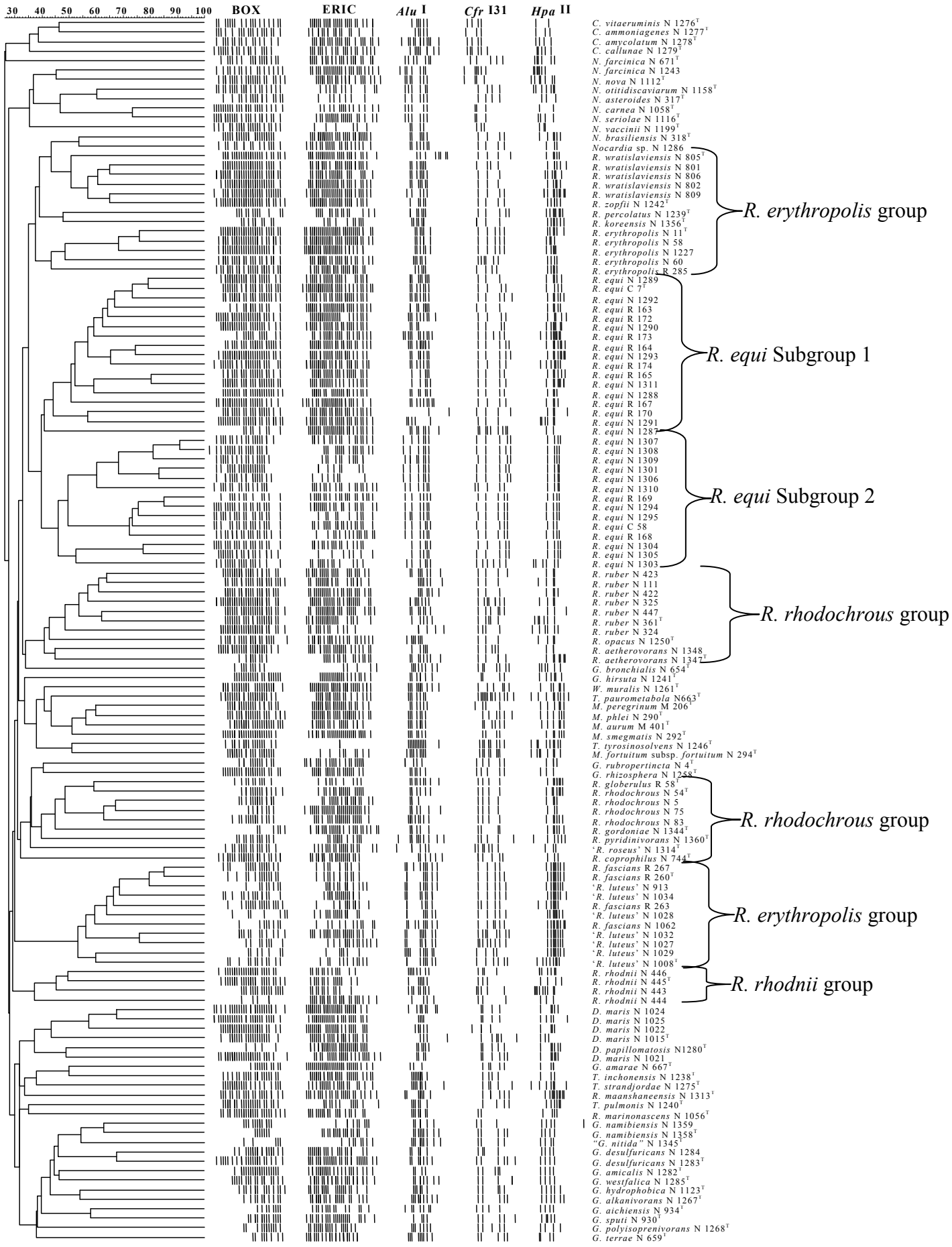
Rhodococcus equi C 7T (X80614)
Rhodococcus equi R 163 (DQ157914)
Segniliparus rotundus JCM 13578T (AY608918)
Smaragdicoccus niigatensis DSM 44881T (AB243007)
Nocardia farcinica DSM 43665T (Z36936)
Nocardia transvalensis DSM 43405T (X80609)
Nocardia asteroides DSM 43757T (X84850)
Nocardia brasiliensis DSM 43758T (X80608)
Nocardia seriolae DSM 44129T (X80592)
Nocardia otitidiscaviarum DSM 43242T (AF430067)
Nocardia pseudobrasiliensis DSM 44290T (AF430042)
Nocardia nova DSM 44481T (AF430028)
Nocardia crassostreae DSM 44597T (Z37989)
Nocardia mexicana DSM 44952T (FR733723)
Williamsia muralis DSM 44343T (Y17384)
Skermania piniformis DSM 43998T (Z35435)
Gordonia bronchialis DSM 43247T (X79287)
Millisia brevis DSM 44463T (AY534742)
Tomitella biformata DSM 45403T (AB491283)
Dietzia maris DSM 43672T (X79290)
Tsukamurella paurometabola DSM 20162T (Z46751)
Mycobacterium tuberculosis ATCC 27294T (X52917)
Amycolicicoccus subflavus DSM 45089 (EF564379)
Hoyosella altamirensis DSM 45258T (FJ179485)
Corynebacterium diphtheriae DSM 44123T (X84248)
Turicella otitidis DSM 8821T (X73976)
R. erythropolis
R. rhodochrous
R. corynebacterioides
R. equi
Nocardia
0.005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BOX
ERIC
Alu I
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Hpa II
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C. ammoniagenes N 1277T
C. amycolatum N 1278T
C. callunae N 1279T
N. farcinica N 671T
N. farcinica N 1243
N. nova N 1112T
N. otitidiscaviarum N 1158T
N. asteroides N 317T
N. carnea N 1058T
N. seriolae N 1116T
N. vaccinii N 1199T
N. brasiliensis N 318T
Nocardia sp. N 1286
R. wratislaviensis N 805T
R. wratislaviensis N 801
R. wratislaviensis N 806
R. wratislaviensis N 802
R. wratislaviensis N 809
R. zopfii N 1242T
R. percolatus N 1239T
R. koreensis N 1356T
R. erythropolis N 11T
R. erythropolis N 58
R. erythropolis N 1227
R. erythropolis N 60
R. erythropolis R 285
R. equi N 1289
R. equi C 7T
R. equi N 1292
R. equi R 163
R. equi R 172
R. equi N 1290
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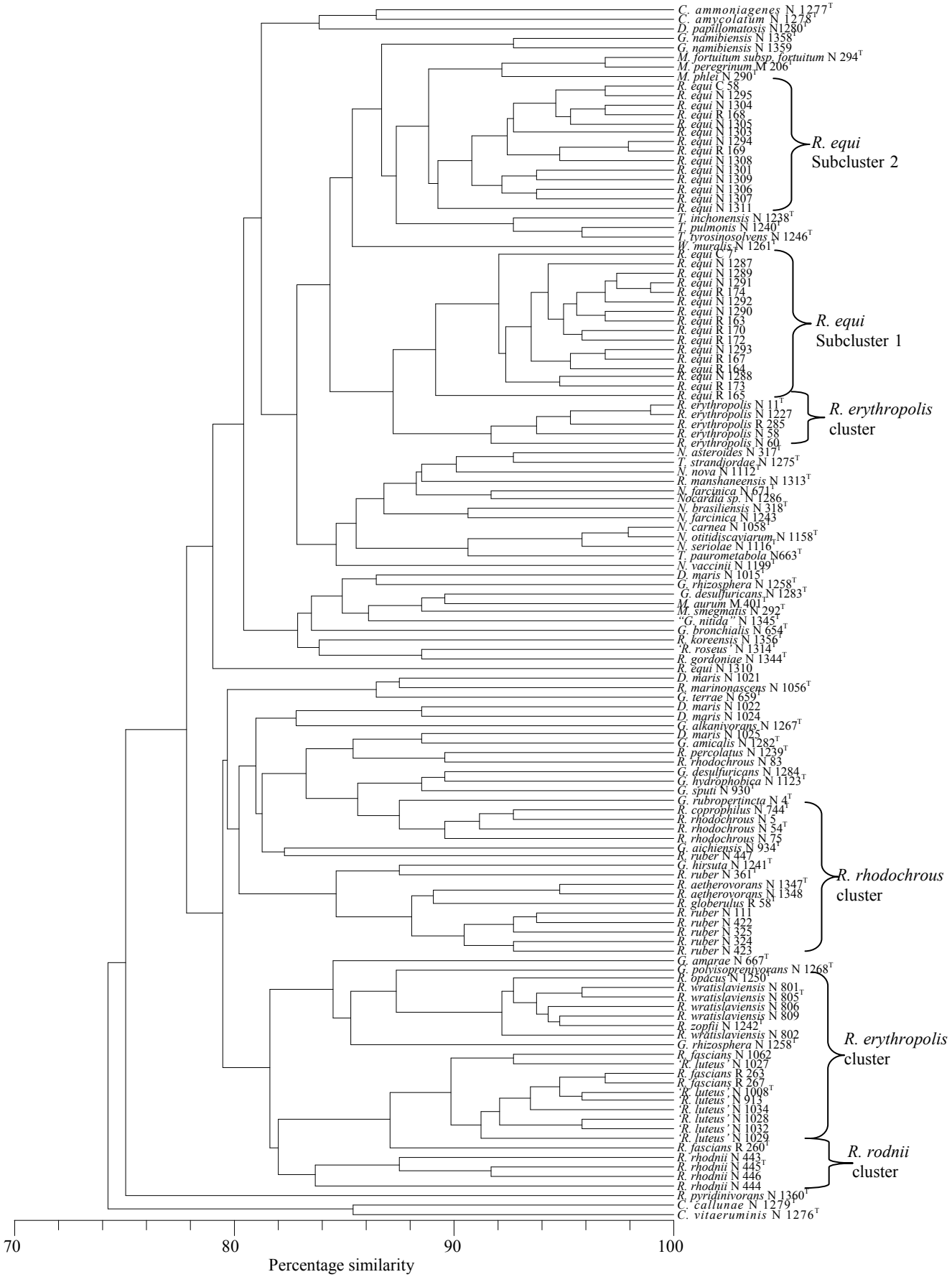
R. equi N 1293
R. equi R 174
R. equi R 165
R. equi N 1311
R. equi N 1288
R. equi R 167
R. equi R 170
R. equi N 1291
R. equi N 1287
R. equi N 1307
R. equi N 1308
R. equi N 1309
R. equi N 1301
R. equi N 1306
R. equi N 1310
R. equi R 169
R. equi N 1294
R. equi N 1295
R. equi C 58
R. equi R 168
R. equi N 1304
R. equi N 1305
R. equi N 1303
R. ruber N 423
R. ruber N 111
R. ruber N 422
R. ruber N 325
R. ruber N 447
R. ruber N 361T
R. ruber N 324
R. opacus N 1250T
R. aetherovorans N 1348
R. aetherovorans N 1347T
G. bronchialis N 654T
G. hirsuta N 1241T
W. muralis N 1261T
T. paurometabola N663T
M. peregrinum M 206T
M. phlei N 290T
M. aurum M 401T
M. smegmatis N 292T
T. tyrosinosolvens N 1246T
M. fortuitum subsp. fortuitum N 294T
G. rubropertincta N 4T
G. rhizosphera N 1258T
R. globerulus R 58T
R. rhodochrous N 54T
R. rhodochrous N 5
R. rhodochrous N 75
R. rhodochrous N 83
R. gordoniae N 1344T
R. pyridinivorans N 1360T
'R. roseus' N 1314T
R. coprophilus N 744T
R. fascians R 267
R. fascians R 260T
'R. luteus' N 913
'R. luteus' N 1034
R. fascians R 263
'R. luteus' N 1028
R. fascians N 1062
'R. luteus' N 1032
'R. luteus' N 1027
'R. luteus' N 1029
'R. luteus' N 1008T
R. rhodnii N 446
R. rhodnii N 445T
R. rhodnii N 443
R. rhodnii N 444
D. maris N 1024
D. maris N 1025
D. maris N 1022
D. maris N 1015T
D. papillomatosis N1280T
D. maris N 1021
G. amarae N 667T
T. inchonensis N 1238T
T. strandjordae N 1275T
R. maanshaneensis N 1313T
T. pulmonis N 1240T
R. marinonascens N 1056T
G. namibiensis N 1359
G. namibiensis N 1358T
"G. nitida" N 1345T
G. desulfuricans N 1284
G. desulfuricans N 1283T
G. amicalis N 1282T
G. westfalica N 1285T
G. hydrophobica N 1123T
G. alkanivorans N 1267T
G. aichiensis N 934T
G. sputi N 930T
G. polyisoprenivorans N 1268T
G. terrae N 659T
R. erythropolis group
R. equi Subgroup 1
R. equi Subgroup 2
R. rhodochrous group
R. rhodochrous group
R. erythropolis group
R. rhodnii group


C. ammoniagenes N 1277T
C. amycolatum N 1278T
D. papillomatosis N1280T
G. namibiensis N 1358T
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M. fortuitum subsp. fortuitum N 294T
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R. equi C 58
R. equi N 1295
R. equi N 1304
R. equi R 168
R. equi N 1305
R. equi N 1303
R. equi N 1294
R. equi R 169
R. equi N 1308
R. equi N 1301
R. equi N 1309
R. equi N 1306
R. equi N 1307
R. equi N 1311
R. equi Subcluster 2
T. inchonensis N 1238T
T. pulmonis N 1240T
T. tyrosinosolvens N 1246T
W. muralis N 1261T
R. equi C 7
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R. equi R 163
R. equi R 170
R. equi R 172
R. equi N 1293
R. equi R 167
R. equi R 164
R. equi N 1288
R. equi R 173
R. equi R 165
R. equi Subcluster 1
R. erythropolis N 11T
R. erythropolis N 1227
R. erythropolis R 285
R. erythropolis N 58
R. erythropolis N 60
R. erythropolis cluster
N. asteroides N 317T
T. strandjordae N 1275T
N. nova N 1112T
R. manshaneensis N 1313T
N. farcinica N 671T
Nocardia sp. N 1286
N. brasiliensis N 318T
N. farcinica N 1243
N. carnea N 1058T
N. otitidiscaviarum N 1158T
N. seriolae N 1116T
T. paurometabola N663T
N. vaccinii N 1199T
D. maris N 1015T
G. rhizosphera N 1258T
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M. aurum M 401T
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G. bronchialis N 654T
R. koreensis N 1356T
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R. gordoniae N 1344T
R. equi N 1310
D. maris N 1021
R. marinonascens N 1056T
G. terrae N 659T
D. maris N 1022
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G. alkanivorans N 1267T
D. maris N 1025
G. amicalis N 1282T
R. percolatus N 1239T
R. rhodochrous N 83
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G. hydrophobica N 1123T
G. sputi N 930T
G. rubropertincta N 4T
R. coprophilus N 744T
R. rhodochrous N 5
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R. ruber N 447
G. hirsuta N 1241T
R. ruber N 361T
R. aetherovorans N 1347T
R. aetherovorans N 1348
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R. ruber N 111
R. ruber N 422
R. ruber N 325
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R. rhodochrous cluster
G. amarae N 667T
G. polyisoprenivorans N 1268T
R. opacus N 1250T
R. wratislaviensis N 801
R. wratislaviensis N 805T
R. wratislaviensis N 806
R. wratislaviensis N 809
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"R. luteus" N 913
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"R. luteus" N 1028
"R. luteus" N 1032
"R. luteus" N 1029
R. fascians R 260T
R. erythropolis cluster
R. rhodnii N 443
R. rhodnii N 445T
R. rhodnii N 446
R. rhodnii N 444
R. rodnii cluster
R. pyridinivorans N 1360T
C. callunae N 1279T
C. vitaeruminis N 1276T
70
80
90
100
Percentage similarity