Transfer of *Propionibacterium innocuum* Pitcher and Collins 1991 to *Propioniferax* gen. nov. as *Propioniferax innocua* comb. nov.

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16S ribosomal DNA analysis indicates that *Propionibacterium innocuum* is a phylogenetic neighbor of *Luteococcus japonicus* and that this pair of organisms branches intermediately between the genus *Propionibacterium* on the one side and the genera *Aeromicrobium* and *Nocardioides* on the other side. Phenotypically, strains of *P. innocuum* differ from species of *Propionibacterium* by exhibiting aerobic growth and possessing arabinose in the cell wall, they differ from species of *Aeromicrobium* and *Nocardioides* by the formation of propionic acid, and they differ from species of *Luteococcus* in morphology. Consequently, *P. innocuum* should not be classified with authentic *Propionibacterium* species, and the transfer of *P. innocuum* Pitcher and Collins 1991 to a new genus, *Propioniferax*, as *Propioniferax innocua* gen. nov., comb. nov. is proposed.

It has been proposed recently by Pitcher and Collins (14) that the species *Propionibacterium innocuum* is an LL-diaminopimelic acid (LL-A₂pm)-containing coryneform bacterium. However, in contrast to the descriptions of most *Propionibacterium* species (2, 4), strains of the species *Propionibacterium innocuum* show aerobic and facultatively anaerobic growth, contain arabinose in cell wall hydrolysates, and do not require blood, serum, or Tween 80 for growth. 16S rRNA analysis placed *Propionibacterium innocuum* as a remotely related member of the genus *Propionibacterium* (14). However, outside reference organisms that would have shown the position of *Propionibacterium innocuum* in relation to other coryneform and actinomycete species were not included in the phylogenetic analysis (14). On the basis of physiological and chemotaxonomic characteristics and a 16S rRNA sequence comparison, we propose the transfer of the species *Propionibacterium innocuum* from the genus *Propionibacterium* to a new genus, *Propioniferax*, as *Propioniferax innocua* gen. nov., comb. nov.

Propionibacterium acidipropionici DSM 4900^T, *Propionibacterium acnes* DSM 1897^T, *Propionibacterium freudenreichii* DSM 20271^T, *Propionibacterium jensenii* DSM 20535^T, *Propionibacterium propionicus* DSM 43307^T, *Propionibacterium thoenii* DSM 20276^T, and *Propionibacterium innocuum* DSM 8251^T (NCTC 11082^T) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. For chemotaxonomic studies these strains were grown in tryptic soy broth (1% casein peptone, 0.5% yeast extract, 1% sodium lactate [pH 7.0]) at 30°C, and cells were harvested in the stationary phase, washed twice with water, and then freeze-dried.

Production of propionic acid from glucose was determined by gas-liquid chromatography of culture supernatants following anaerobic growth in tryptic soy broth for 8 days.

Analyses of cell wall sugars (20), amino acid composition and peptidoglycan structure (16, 20), and lipids (9) were performed as described previously.

Comparison of 16S rRNAs was carried out as follows. All sequences were obtained from the DDBJ, GenBank, and the EMBL Data Library under the following accession numbers:

Propionibacterium acidipropionici DSM 20272, X53221; *Propionibacterium acnes* DSM 1897^T, X53218; *Propionibacterium freudenreichii* DSM 20271^T, X53217; *Propionibacterium jensenii* DSM 20535^T, X53219; *Propionibacterium propionicus* DSM 43307^T, X53216; *Propionibacterium thoenii* DSM 20276^T, X53220; *Arthrobacter globiformis*, M23411; *Micrococcus luteus*, M38242; *Terrabacter tumescens* NCIB 8914^T, X53215; *Sporichthya polymorpha* DSM 46113, X72377; *Streptomyces griseus* KCTC 9080, X61478; *Nocardioides fastidiosa* NCIB 12713^T, X53189; *Aeromicrobium erythreum* NRRL B-3381^T, M37200; *Nocardioides jensenii* DSM 20641^T, X53214; *Nocardioides luteus* NCIB 11455^T, X53212; *Nocardioides albus* DSM 43109^T, X53211; and *Luteococcus japonicus* IFO 12422^T, D21245. The sequences of *Nocardioides simplex* ATCC 6946^T, *Bacillus subtilis* 168, and *Propionibacterium innocuum* NCTC 11082^T were taken from references 3, 5, and 14, respectively. DNA sequences were aligned by using the ODEN system (7). Nucleotide substitution rate values were calculated (8), and a phylogenetic tree was constructed by the neighbor-joining method (15). The topology of the tree was evaluated by bootstrap analysis of the sequence data using Clustal V software (6). The search for *Propionibacterium innocuum* 16S ribosomal DNA (rDNA)-specific nucleotides was carried out by using the ae2 editor included in the Ribosomal Database Project package (10).

16S rRNA sequence analysis has substantially facilitated the classification of actinomycete genera and species (17). Comparison of 16S rRNAs of authentic *Propionibacterium* species showed sequence similarities ranging between 94.3 and 98.8%, except for *Propionibacterium innocuum*, for which values for similarity with other *Propionibacterium* species ranged between 91.7 and 93.3% (Table 1). Without the comparison of reference organisms, *Propionibacterium innocuum* appears to be the most distantly related member of its genus, as previously suggested by Pitcher and Collins (14). If, however, the closest relatives of the genus *Propionibacterium*, i.e., members of the genera *Aeromicrobium* and *Nocardioides* (3, 11), are included in the analyses, *Propionibacterium innocuum* does not branch with the propionibacteria but rather with *L. japonicus* (18) (94.5% similarity), and this forms a separate line of descent. *Propionibacterium innocuum* can be characterized by 16 and 7 unique nucleotides that are not found in the 16S rDNAs of species of *Propionibacterium* and the *Aeromicrobium-Nocardioides* cluster (Table 2), respectively. The 16S rDNA relationship

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TABLE 1. Similarity values for the 16S rDNA sequences of *Propioniferax*, *Propionibacterium*, *Luteococcus*, *Nocardioides* and *Aeromicrobium* species

Strain	% Similarity to:													
	<i>Propioniferax innocua</i>	<i>Propionibacterium propionicus</i>	<i>Propionibacterium acidipropionici</i>	<i>Propionibacterium jensenii</i>	<i>Propionibacterium acnes</i>	<i>Propionibacterium freudenreichii</i>	<i>Propionibacterium thoenii</i>	<i>L. japonicus</i>	<i>N. luteus</i>	<i>N. albus</i>	<i>N. simplex</i>	<i>N. jensenii</i>	<i>N. fastidiosa</i>	<i>A. erythreum</i>
<i>Propioniferax innocua</i>														
<i>Propionibacterium propionicus</i>	93.2													
<i>Propionibacterium acidipropionici</i>	92.2	95.9												
<i>Propionibacterium jensenii</i>	92.8	94.8	97.3											
<i>Propionibacterium acnes</i>	91.7	95.0	95.4	96.1										
<i>Propionibacterium freudenreichii</i>	93.3	94.5	94.3	94.8	94.4									
<i>Propionibacterium thoenii</i>	92.7	95.0	97.3	98.8	95.7	94.8								
<i>L. japonicus</i>	94.5	93.2	91.7	92.3	92.1	92.9	92.4							
<i>N. luteus</i>	93.2	92.0	91.8	92.1	91.2	91.8	92.3	93.2						
<i>N. albus</i>	93.4	91.6	91.5	91.4	90.6	91.0	91.6	92.3	98.8					
<i>N. simplex</i>	92.9	92.9	91.6	92.0	91.8	92.1	92.3	92.0	96.7	96.5				
<i>N. jensenii</i>	93.7	92.2	91.2	91.6	91.0	91.5	92.0	93.3	97.7	97.0	96.1			
<i>N. fastidiosa</i>	93.9	92.7	91.4	91.7	91.2	91.2	92.0	93.4	96.3	96.0	94.6	95.9		
<i>A. erythreum</i>	93.9	93.2	92.2	92.3	91.7	92.4	92.7	93.4	95.9	95.3	94.9	96.0	98.2	
<i>T. tumescens</i>	92.2	92.2	91.1	92.1	91.2	91.8	92.3	92.8	93.3	92.7	93.2	93.5	92.6	93.2
<i>M. luteus</i>	91.2	90.7	90.5	90.9	89.9	91.1	91.2	90.7	92.3	92.4	92.4	92.4	93.2	94.2
<i>A. globiformis</i>	91.1	91.2	90.1	90.6	90.3	90.9	91.0	90.7	92.1	92.1	92.7	91.8	92.3	92.7
<i>S. griseus</i>	90.7	90.9	89.8	90.1	89.3	90.7	90.5	90.6	91.5	91.0	90.7	92.0	91.0	92.2
<i>S. polymorpha</i>	92.2	92.0	90.3	90.6	89.6	90.9	91.0	92.0	92.8	92.3	92.9	93.2	92.8	93.9
<i>B. subtilis</i>	83.7	82.0	82.2	82.1	81.5	82.3	82.0	83.3	82.8	82.8	83.4	83.1	82.7	83.1

TABLE 2. 16S rDNA signature nucleotides showing the distinctness of the *Propioniferax*-*Luteococcus* cluster from the *Propionibacterium* and *Aeromicrobium*-*Nocardioides* lines of descent

Position of signature nucleotide(s) ^a	16S rDNA sequence in:		
	<i>Propionibacterium</i> spp.	<i>Propioniferax</i> - <i>Luteococcus</i> cluster	<i>Aeromicrobium</i> - <i>Nocardioides</i> cluster
322-343	U-G	C-G	C-G
334	U	U	C
413-441	C-G	Y-G	A-U
515	U	C	C
598	G	U	U
599-654	A-U	G-C	G-C
660	U	A	A(U)
693	G	G	U
794	C	C	A
832-859	A-U	A-U	G-C
867	U	C	C
1006-1051	G-C	A-U	A-U
1129-1159	A-U	U-A	U-A
1166	C	U	U
1263	U	C	C
1261-1290	A-U	G-C	G-C

^a Position (by *Escherichia coli* numbering) (1) at which the 16S rDNA sequences of *Propioniferax* and *Luteococcus* have a common composition that differs from the common composition of *Propionibacterium* sequences and/or that of *Aeromicrobium*-*Nocardioides* cluster sequences.

between *Propionibacterium innocuum* and *L. japonicus* is reflected by the presence of several nucleotides which they share either with propionibacteria or with members of the *Aeromicrobium*-*Nocardioides* cluster (Table 2).

As described above, *Propionibacterium innocuum* shares certain properties with neighboring taxa, such as peptidoglycan containing LL-A₂pm and glycine forming the interpeptide bridge (*Propionibacterium propionicus* is the only species of this group that contains a glycine residue in position 1 of the peptide subunit, whereas all other species contain alanine). *Propionibacterium innocuum* resembles authentic species of *Propionibacterium* (12) in that it contains high levels of iso-branched-chain and anteiso-branched-chain compounds (iso-15:0, 22.7%; iso-16:0, 12.1%; iso-17:0, 5.9%; anteiso-15:0, 37.3%; and anteiso-17:0, 11.1%). In contrast, members of the genera *Aeromicrobium* and *Nocardioides* are characterized by complex mixtures of straight-chain saturated, monounsaturated, and iso-branched, anteiso-branched, and 10-methyl-branched fatty acids (11, 13, 19).

Biochemically, *Propionibacterium innocuum* differs from *Aeromicrobium* species and their relatives by producing propionic acid from glucose under facultatively anaerobic conditions (at a yield of ca. 75% and with no acetic acid production), and it differs from authentic propionibacteria by its aerobic and facultatively anaerobic growth, the presence of arabinose in its cell wall hydrolysates, and the fact that it does not require blood, serum, or Tween 80 for growth (Table 3).

On the basis of physiological and phylogenetic data, we propose that *Propionibacterium innocuum* be transferred to the

TABLE 3. Characteristics distinguishing the genera *Propioniferax*, *Luteococcus*, *Propionibacterium*, and the *Aeromicrobium-Nocardioides* cluster

Characteristic	<i>Propioniferax</i> spp.	<i>Luteococcus</i> spp.	<i>Propionibacterium</i> spp.	<i>Aeromicrobium-Nocardioides</i> cluster
Morphology	Rod	Coccus	Rod	Rod or hypha
Respiration	Aerobe	Aerobe	Anaerobe	Aerobe
Principal cell wall neutral sugars	Arabinose, mannose	Arabinose, glucose	(Galactose), ^a (glucose), (rhamnose), mannose	Galactose, (glucose), (rhamnose), (mannose)
Hydrolysis of starch	+	+	—	+
Formation of propionic acid from glucose	+	+	+	—
Requirement of blood, serum, or Tween 80	—	—	+	—
Amino acid in peptidoglycan ^b				
Diamino acid	LL-A ₂ pm	LL-A ₂ pm	LL-A ₂ pm, meso-A ₂ pm, or Lys	LL-A ₂ pm
Amino acid in position 1	Gly	ND ^c	Ala	Ala
Menaquinone composition	MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₄) or MK-8(H ₄)

^a Parentheses indicate that a compound may or may not be present.

^b Lys, lysine; Gly, glycine; Ala, alanine.

^c ND, not determined.

genus *Propioniferax* gen. nov. as *Propioniferax innocua* (Pitcher and Collins) comb. nov.

The description of *Propioniferax* gen. nov. is taken from Pitcher and Collins (14) and our own data. *Propioniferax* (Pro.pi.on.i.fe'rax. M.L. n. *acidum propionicum*, propionic acid; L. adj. *ferax*, fertile; M.L. n. *propioniferax*, propionic acid-producing). Cells are gram-positive, non-acid fast, non-motile, nonsporing, pleomorphic rods appearing in clusters and V forms. Colonies are white, shining, and convex to domed. The optimum growth temperature is approximately 37°C. Grows at 10 and 40°C. Facultatively anaerobic but luxuriant growth occurs aerobically (approximately 0.5 to 3 mm of growth on nutrient or horse blood agar at 37°C for 2 days). Catalase and oxidase positive. Cell wall contains LL-A₂pm, arabinose, and mannose but not galactose. Glycine forms the interpeptide bridge of peptidoglycan. Mycolic acids are not present. Major fatty acids are anteiso-15:0 and iso-15:0. The major respiratory quinone is MK-9(H₄). The G+C content of the DNA is 59 to 63 mol%. The genus *Propioniferax* is a member of the *Propionibacterium-Luteococcus-Aeromicrobium-Nocardioides* line of descent within the radiation of the order *Actinomycetales*. Its phylogenetic neighbor is *L. japonicus* (Fig. 1).

The type species is *Propioniferax innocua*.

The description of *Propioniferax innocua* comb. nov. is the same as that published for *Propionibacterium innocuum* (Pitcher and Collins 1991) (14). *innocua* (in.noc'u.a. L. neut. adj. *innocua*, harmless.) Cells are gram-positive, non-acid fast, nonmotile, nonsporing, pleomorphic rods appearing in clusters and V forms. Colonies are white, shining, and convex to domed. The optimum growth temperature is approximately 37°C. Grows at 10 and 40°C. Facultatively anaerobic, but substantial growth occurs aerobically (approximately 0.5 to 3 mm of growth on nutrient or horse blood agar for 2 days). Catalase and oxidase positive. The principal carboxylic acid produced from glucose is propionic acid. Glucose, sucrose, maltose, trehalose, fructose, mannose, and glycerol are fermented. Inositol, arabinose, adonitol, salicin, erythritol, mannitol, cellobiose, dulcitol, raffinose, lactose, sorbose, sorbitol, and rhamnose are not utilized, and galactose utilization is variable. Gelatin is hydrolyzed. Growth occurs in the presence of 7.5% NaCl but not in 10% NaCl. Reduces nitrate. Hydrolyzes urea and starch. Growth is not stimulated by Tween 80, but Tween 80 is hydrolyzed. The following tests are negative:

arginine dihydrolase, esculin hydrolysis, glutamate oxidation, arginine, lysine and ornithine decarboxylase, phenylalanine deaminase, citrate and malonate utilization, indole, H₂S, and decomposition of tyrosine and xanthine.

Cell wall contains LL-A₂pm, arabinose, and mannose but not galactose. Mycolic acids are not present. The major respiratory quinone is MK-9(H₄). The G+C content of the DNA is 59 to

FIG. 1. Phylogenetic tree showing the relationship among *Propioniferax innocua* and related species. The tree was constructed by using the nucleotide substitution rate (K_{nuc}) values.

63 mol%, and that of the type strain is 62 mol%. Habitat: human epidermal surface.

The type strain is NCTC 11082 (DSM 8251).

REFERENCES

1. Brosius, J., J. L. Palmer, J. P. Kennedy, and H. F. Noller. 1978. Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **75**:4801–4805.
2. Charfreitag, O., M. D. Collins, and E. Stackebrandt. 1988. Reclassification of *Arachnia propionica* as *Propionibacterium propionicus* comb. nov. Int. J. Syst. Bacteriol. **38**:354–357.
3. Collins, M. D., M. Dorsch, and E. Stackebrandt. 1989. Transfer of *Pimelobacter tumescens* to *Terrabacter* gen. nov. as *Terrabacter tumescens* comb. nov. and of *Pimelobacter jensenii* to *Nocardioides* as *Nocardioides jensenii* comb. nov. Int. J. Syst. Bacteriol. **39**:1–6.
4. Cummins, C. S., and J. L. Johnson. 1986. Genus I. *Propionibacterium* Orla-Jensen 1909, 337^{AL}, p. 1346–1353. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. The Williams and Wilkins Co., Baltimore.
5. Green, C. J., G. C. Stewart, M. A. Hollis, B. S. Vold, and K. F. Bott. 1985. Nucleotide sequence of the *Bacillus subtilis* ribosomal RNA operon, *rmB*. Gene **37**:261–266.
6. Higgins, D. R., A. J. Bleasby, and R. Fuchs. 1992. Clustal V: improved software for multiple sequence alignment. Comput. Appl. Biosci. **8**:189–190.
7. Ina, Y. 1991. Molecular evolutionary analysis system for DNA and amino acid sequences (ODEN), version 1.1. DNA Data Bank of Japan, DNA Research Center, National Institute of Genetics, Mishima, Japan.
8. Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16**:111–120.
9. Kroppenstedt, R. M. 1985. Fatty acid and menaquinone analysis of actinomycetes and related organisms, p. 173–199. In M. Goodfellow and D. E. Minnikin (ed.), *Chemical methods in bacterial systematics*. Academic Press, London.
10. Larsen, N., G. J. Olsen, B. L. Maidak, M. J. McCaughey, R. Overbeek, T. J. Macke, T. L. Marsh, and C. R. Woese. 1993. The ribosomal database project. Nucleic Acids Res. **21**:3021–3023.
11. Miller, E. S., C. R. Woese, and S. Brenner. 1991. Description of the erythromycin-producing bacterium *Arthrobacter* sp. strain NRRL B-3381 as *Aeromicrobium erythreum* gen. nov., sp. nov. Int. J. Syst. Bacteriol. **41**:363–368.
12. Moss, C. W., V. R. Dowell, D. Farshtchi, L. J. Raines, and W. B. Cherry. 1969. Cultural characteristics and fatty acid composition of propionibacteria. J. Bacteriol. **97**:561–570.
13. O'Donnell, A., M. Goodfellow, and D. E. Minnikin. 1982. Lipids in the classification of *Nocardioides*: reclassification of *Arthrobacter simplex* (Jensen) Lochhead in the genus *Nocardioides* (Prauser) emend. O'Donnell et al. as *Nocardioides simplex* comb. nov. Arch. Microbiol. **133**:323–329.
14. Pitcher, D. G., and M. D. Collins. 1991. Phylogenetic analysis of some LL-diaminopimelic acid-containing coryneform bacteria from human skin: description of *Propionibacterium innocuum* sp. nov. FEMS Microbiol. Letters **84**:295–300.
15. Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406–425.
16. Schleifer, K. H., and O. Kandler. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol. Rev. **36**:407–477.
17. Stackebrandt, E. 1991. Aspects on the evolution and phylogeny of the actinomycetes. Actinomycetologica **5**:56–63.
18. Tamura, T., M. Takeuchi, and A. Yokota. 1994. *Luteococcus japonicus* gen. nov., sp. nov., a new gram-positive coccus with LL-diaminopimelic acid in the cell wall. Int. J. Syst. Bacteriol. **44**:348–356.
19. Yano, I., Y. Furukawa, and M. Kusunose. 1971. 2-Hydroxy fatty acid containing phospholipid of *Arthrobacter simplex*. Biochim. Biophys. Acta **210**:105–115.
20. Yokota, A., M. Takeuchi, and N. Weiss. 1993. Proposal of two new species in the genus *Microbacterium*: *Microbacterium dextranolyticum* sp. nov. and *Microbacterium aurum* sp. nov. Int. J. Syst. Bacteriol. **43**:549–554.