Establishment of *Kineococcus*, *Luteococcus*, *Catenuloplanes* and *Couchioplanes* as New Actinomycete Genera Including New Species, and Their Relationships with Related Organisms

Tomohiko Tamura

Institute for Fermentation, Osaka, 2-17-85 Jusohonmachi, Yodogawa-ku, Osaka 532, Japan

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Taxonomy of actinomycetes is based on morphological, physiological and chemotaxonomic characteristics until now. Especially, chemotaxonomy has proved to be very useful in providing characteristics that distinguish the level of the genus of actinomycetes from neighboring microorganisms which have no morphological characteristics. Recently, taxonomic criteria of classification for the actinomycetes have been changed by introduction and application of new techniques such as DNA relatedness on the level of the species and 16S rRNA sequence on the levels of the genus and the family. Phylogenetic analysis on the basis of 16S rRNA sequence is used for distinguishing the relationship among these taxa on the level of the genus and the family.

We have proposed some new genera and new species of actinomycetes and related bacteria using new taxonomic techniques, and attempted reconstruction of the taxa.

Kineococcus gen. nov.

Among genera *Micrococcus*¹⁾, *Deinococcus*²⁾, *Planococcus*¹⁾, *Marinococcus*³⁾, *Sporosarcina*⁴⁾, and *Salinicoccus*⁵⁾ which had been previously described as genera of aerobic, Gram-positive cocci, *Planococcus*, *Marinococcus*, and *Sporosarcina* species and some strains of *Micrococcus agilis*⁶⁾ are known as motile bacteria.

During the course of our taxonomic study of motile actinomycetes from natural sources, a strain was isolated from soil in India. The strain,

RA 333^T (T=type strain) was a Gram-positive coccus with a high guanine-plus-cytosine (G+C) content of the DNA, *meso*-diaminopimelic acid (DAP) in the cell wall, and motility. The strain has the following chemotaxonomic characteristics: menaquinone MK-9(H₂); the G+C content of the DNA is 73.9 mol%; *meso*-diaminopimelic acid, glutamic acid, and alanine in a molar ratio of ca. 1:1:2 (type A1γ); and arabinose and galactose in the cell wall; and lacked mycolic acid (Table 1). It was clarified that two kinds of sugar are contained in the cell wall as an arabinogalactan polymer. The menaquinone and cellular fatty acid compositions, the G+C content of the DNA, and the presence of motility in strain RA 333^T most closely resemble the characteristics of *Micrococcus agilis*⁶⁾. However, these organisms are different, as *M. agilis* contains type A4α peptidoglycan, has no characteristic sugar in its cell wall, and produces a different polar lipid pattern.

Thus, on the basis of biochemical and chemical criteria, this isolate can be distinguished readily from all previously described aerobic, Gram-positive cocci. Therefore, we formally proposed that strain RA 333^T should be classified in a new genus, *Kineococcus*, as *Kineococcus aurantiacus* gen. nov., sp. nov. (the type strain RA 333 = IFO 15268)³⁵⁾.

Luteococcus gen. nov.

The following five genera of gram-positive cocci with a high G+C content have of the DNA been described previously: *Micrococcus*⁷⁾,

Table 1. Chemotaxonomic characteristics of genus *Kineococcus*.

Characteristic	
Quinone composition	MK-9(H ₂)
Cellular fatty acid	anteiso-C _{15:0} (88.7%) anteiso-C _{17:0} (1.7%)
Peptidoglycan amino acid (molar ratio)	
<i>meso</i> -diamino pimelic acid	0.56
alanine	2.00
glutamic acid	0.92
Cell wall sugar (molar ratio)	
arabinose	1.10
galactose	1.00
Mycolic acid	absent
G + C content (mol%)	73.7

*Deinococcus*²⁾, *Stomatococcus*⁸⁾, and *Pelczaria*⁹⁾. However, Gram-positive cocci with a high-G+C-content and containing LL-DAP have not been known. The genus *Sarcina*⁷⁾ is the only coccus with LL-DAP in the cell wall, but this genus has a low G+C content of the DNA and is a strict anaerobe. As Gram-positive rod-shaped

bacteria with a high G+C content, the genera *Nocardioides*¹⁰⁾, *Aeromicrobium*¹¹⁾, *Terrabacter*¹²⁾, *Propionibacterium*¹³⁾, *Sporichthya*¹⁴⁾ and *Streptomyces*¹⁵⁾ are known.

Gram-positive cocci "*Micrococcus aurantiacus*" IFO 12422 and *Micrococcus* sp. IFO 15385 (=CCM 2142) were isolated from soil on

Table 2. Chemotaxonomic characteristics of genus *Luteococcus*.

Characteristic	
Quinone composition	MK-9(H ₄)
Cellular fatty acid	C _{16:1} C _{17:1} C _{18:1}
Peptidoglycan amino acid (molar ratio)	
LL-diaminopimelic acid	0.72
alanine	2.00
glycine	1.03
glutamic acid	0.66
Characteristic cell wall sugar	arabinose
Mycolic acid	absent
G + C content (mol%)	67

Tokara island and from water for brewing "Miyamizu" in Japan, respectively¹⁶, and have been maintained in the culture collections of IFO and Czech Collection of Microorganisms (CCM). *Micrococcus* species contain lysine in their cell walls, but strains IFO 12422 and IFO 15385 contain LL-diaminopimelic acid (LL-DAP) instead of lysine. Therefore, the taxonomic position of these strains has remained uncertain.

Strains IFO 12422 and IFO 15385 have the following chemotaxonomic characteristics: menaquinone MK-9(H₄); the G+C content of the DNA is 67 mol%; LL-diaminopimelic acid, alanine, glycine and glutamic acid in a molar ratio of ca. 1:2:1:1 (type A3γ); and lacked mycolic acid (Table 2). The DNA-DNA hybridization study indicated that strain IFO 12422^T and IFO 15385 belong to the same species. The chemotaxonomic characteristics of strains IFO 12422^T and IFO 15385 closely resemble the characteristics of *Nocardioide*¹⁷, *Aeromicrobi-*

*um*¹¹, *Terrabacter*¹² and *Propionibacterium*¹³, but the fatty acid compositions and morphological characteristics are different. With respect to menaquinone composition, major menaquinone of strains of IFO 12422^T, IFO 15385, *Nocardioide fastidiosa*, *Aeromicrobium erythreum* and species of *Propionibacterium* is MK-9(H₄), but that of *Terrabacter tumescens* and species of *Nocardioide* except for *N. fastidiosa* are MK-8(H₄) (*N. fastidiosa* was transferred to *Aeromicrobium* as *Aeromicrobium fastidiosum*¹⁸).

Thus, on the basis of biochemical and chemical criteria and phylogenetic analysis, strains IFO 12422^T and IFO 15385 can be readily distinguished from all previously described Gram-positive cocci and in our opinion warrant a new taxon. Therefore, we proposed that strains IFO 12422^T and IFO 15385 be classified in a new genus, *Luteococcus*, as *Luteococcus japonicus* gen. nov., sp. nov.²⁰

The comparative analyses of 16S rRNA

Fig. 1. Phylogenetic tree showing the relationship among *Kineococcus*, *Luteococcus*, and related bacteria.

sequences of the new cocci were carried out^{19,20}. The partial 16S rRNA sequence indicated that *Luteococcus* and *Kineococcus* represented a distinct line of descent among the Gram-positive bacteria with a high G+C content of the DNA (Fig. 1, 2).

Among organisms tested, *L. japonicus* turned out to be very close to *Propioniferax innocua*²¹ which was rod-shape bacteria (Fig. 2). As shown in Fig. 1, the comparative analysis of 16S rRNA sequences have suggested that the genus *Micrococcus* was more closely related to the genus *Arthrobacter* with rod-shape than to the other genera of Gram-positive cocci^{11,22}. The same relationship between *Deinococcus* (coccal-shape) and *Thermus* (rod-shape) was indicated²³. These genera form a phylum distinct from the phylum of Gram-positive bacteria²⁴. These results show that the classification on the basis of 16S rRNA sequences does not support the classification by morphological differences such as coccal and rod shapes in

Gram-positive bacteria having the high G+C content of the DNA.

Catenuloplanes gen. nov.

Celmer *et al.*²⁵ described two actinomycete strains designated N381-16^T and N406-14 that produce the antibiotics CP-5415 and CP-5416. These two strains were deposited in the American Type Culture Collection as strains ATCC 31637^T and ATCC 31638, respectively. Since these strains produced straight chains of spores on their aerial mycelia and the spores showed motility when they were suspended in water, they were placed in a new genus, "*Catenuloplanes*", and gave them the name "*Catenuloplanes japonicus*"²⁵.

In the course of isolating microorganisms that are rarely occurring members of genera belonging to the order *Actinomycetales*, 11 unusual filamentous bacteria were isolated from soils in Japan, India, Philippines and Nepal. These organisms showed morphological, physi-

Fig. 2. Phylogenetic tree showing the relationship among *Luteococcus*, *Propioniferax*, *Propionibacterium*, *Nocardioideis*, *Aeromicrobium*, and related bacteria.

ological, and chemotaxonomic characteristics that fell into those of the order *Actinomycetales*. They had type VI cell walls, lacked mycolic acids, formed motile arthrospores, and contained L-lysine instead of diaminopimelic acid (DAP) in their cell wall and MK-9(H₈) and MK-10(H₈) as their respiratory quinones (Table 3). The genera *Sporichthya*¹⁴⁾, *Actinosynema*²⁶⁾, and *Actinokineospira*²⁷⁾ are previously described as genera with motile arthrospore²⁸⁾. However, our isolates are taxonomically distinct not only from the members of these genera but also from all the known members of the order *Actinomycetales*. Therefore, the name *Catenuloplanes* gen. nov. was proposed²⁹⁾.

The strains could be divided into six groups on the basis of DNA relatedness data²⁹⁾. The groupings correlated well with some characteristics among groups differed and the chemotaxonomic characteristics, the quantitative fatty acid data and difference of whole cell sugar patterns²⁹⁾. Differential characteristics of these six groups are summarized in Table 4. Therefore, on the basis of their cultural, physiological, biochemical and chemotaxonomic charac-

teristics together with DNA-DNA similarity values, these strains can readily be distinguished from each other.

Therefore, we propose that the strains of these groups be classified in the genus *Catenuloplanes* as follows: *C. japonicus* sp. nov. (type strain, N381-16 = IFO 14176^T) for strain N381-16; *Catenuloplanes niger* sp. nov. (type strain, N406-14 = IFO 14177) for strain N406-14; *Catenuloplanes indicus* sp. nov. (type strain, RA328 = IFO 15575) for strains RA327, RA328 and RA329; *Catenuloplanes atrovinosus* sp. nov. (type strain, RA332 = IFO 15579) for strains RA330, RA331 and RA332; *Catenuloplanes castaneus* sp. nov. (type strain, RA344 = IFO 15584) for strains RA336, RA337, RA338 and RA344; *Catenuloplanes nepalensis* sp. nov. (type strain, RA343 = IFO 15583) for strain RA343^{29,36)}.

Couchioplanes gen. nov.

During our taxonomic study of motile actinomycetes isolated from natural sources, strain RA 335 was isolated from soil in Japan. It produces zoospores, has L-lysine in its cell wall,

Table 3. Chemotaxonomic characteristics of genus *Catenuloplanes*.

Characteristics	
G+C content of DNA (mol%)	71-73
Phospholipid type	PIII
Major menaquinone	MK-9(H ₈), MK-10(H ₈)
Amino acid composition of cell walls	glycine, glutamic acid, alanine, serine, lysine
Characteristics sugar in Whole-cell	xylose, mannose, glucose, (galactose)
Major fatty acid	C _{18:1} , anteiso-C _{17:0} , C _{16:0}
Micolic acids	absent
Glycolate test	positive

Table 4. Differential characteristics among group of *Catenuloplanes* species.

	<i>C. japonicus</i> N381-16T	<i>C. niger</i> N406-14	<i>C. indicus</i> RA 327, 328, 329	<i>C. atrovinosus</i> RA330, 331, 332	<i>C. castaneus</i> RA336, 337, 338, 344	<i>C. nepalensis</i> RA343
Growth at 37°C	-	+	+	+	+	-
Soluble pigment in						
ISP medium 4	pale brown	absent	absent	dark vinaceous	absent	absent
ISP medium 6	absent	black	absent	black	absent	absent
ISP medium 7	black	black	absent	black	absent	absent
Bennett's agar	pale brown	pale brown	absent	absent	absent	absent
Maltose Bennett's agar	pale brown	pale brown	absent	absent	pale brown	absent
Nutrient agar	absent	pale brown	absent	pale brown	absent	absent
Color of colony						
ISP medium 4	lt melon yellow	light apricot	lt melon yellow	rose mauve	lt orange	light apricot
ISP medium 5	melon yellow	yellow maple	melon yellow	redwood	deep brown mahogany	orange
Utilization of						
Raffinose	+	+	-	-	-	-
D-Mannitol	+	+	-	+	+	+
Growth at 2% NaCl	-	-	+	+	-	-
Sensitivity against						
penicillin	-	-	+	-	-	-
cephaloridine	+	-	+	-	+	-
cephalexin	+	-	±	-	+	+
benzylpenicillin	+	-	+	-	-	-
Galactose in whole cell sugar	absent	absent	absent	present	absent	absent
Major cellular fatty acid	C _{18:1} , C _{16:0}	C _{18:1} , anteiso- C _{17:0} , C _{16:0}	C _{18:1} , anteiso- C _{17:0} , C _{17:0}	anteiso-C _{17:0} , C _{18:1}	C _{18:1} , C _{16:0}	C _{18:1} , C _{16:0}

a +, growth; -, no growth.

Table 5. Chemotaxonomic characteristics of genus *Couchioplanes*.

Characteristics	
G+C content of DNA (mol%)	70-72
Phospholipid type	PII
Major menaquinone	MK-9(H ₄), MK-10(H ₄)
Amino acid composition of cell walls	glutamic acid, serine, glycine, alanine, lysine
Whole-cell sugar	arabinose, xylose, galactose, glucose, mannose
Major fatty acid	iso-C _{16:0} , anteiso-C _{17:0} , anteiso-C _{15:0}
Mycolic acids	absent
Glucolate test	positive

and forms mature mycelium that changes from orange to dark blue. *Actinoplanes caeruleus* described by Horan and Brodsky³⁰, and "*Actinoplanes azureus*"²⁵) are known to have the same characteristics. *Actinoplanes* species other than *A. caeruleus* contain meso-diaminopimelic acid in their cell walls³¹), but *A. caeruleus* and "*A. azureus*" have lysine instead of meso-diaminopimelic acid in their cell walls³⁰). Therefore, Stackebrandt and Kroppenstedt³²) and Goodfellow *et al.*³³) insisted that *A. caeruleus* should not be included in the genus *Actinoplanes*. Thus, the taxonomic position of *A. caeruleus* and "*A. azureus*" has remained uncertain.

We studied the taxonomic position of these two species and isolate RA 335. These organisms are motile arthrospore-bearing actinomycetes that contain MK-9(H₄) and have lysine and glycine in their cell walls (wall chemotype VI) (Table 5) and therefore differ from the previously described members of the motile arthrospore-bearing actinomycete genera *Sporichthya*¹⁴), *Actinosynnema*²⁶), *Actinokineospora*²⁷), and *Catenuloplanes*²⁹).

Physiological and biochemical characteristics, and DNA-DNA similarity values suggest that "*A. azureus*" should be classified as a

subspecies of *A. caeruleus*. The subspecies can be differentiated on the basis of characteristics summarized in Table 6.

Based on the characterization and classification of *A. caeruleus*, "*A. azureus*" and an isolate RA 335, we proposed that these organisms should be included in a new genus, *Couchioplanes*, as members of *Couchioplanes caeruleus* comb. nov., which is divided into two subspecies, *Couchioplanes caeruleus* subsp. *caeruleus* subsp. nov. and *Couchioplanes caeruleus* subsp. *azureus* subsp. nov.³⁷).

The sequences of 16S rRNA of *C. japonicus* IFO 14176^T and *Couchioplanes caeruleus* subsp. *caeruleus* IFO 13939^T were determined. A phylogenetic tree derived from the sequences is shown in Fig. 3. *C. japonicus* IFO 14176^T and *C. caeruleus* subsp. *caeruleus* IFO 13939^T could be separated from taxonomic neighbors, *Actinoplanes*, *Dactylosporangium*, *Catenuloplanes* and other actinomycete taxa by phylogenetic analysis (Fig. 3).

Since *Catenuloplanes* and *Couchioplanes* contain L-lysine instead of meso-diaminopimelic acid in their cell walls and produce spores within the mycelia arranged in chains instead of enclosed in sporangial walls, they differ from

Table 6. Differential characteristics among genus *Couchioplanes*.

Organism	<i>C. caeruleus</i> subsp. <i>caeruleus</i> (<i>A. caeruleus</i> , isolate RA 335)	<i>C. caeruleus</i> subsp. <i>azureus</i> (<i>A. azureus</i>)
Utilization of:		
Rhamnose	+	-
Mannitol	+	±
Production of pigment on:		
Yeast extract-malt extract agar	-	+
Glycerol-asparagine agar	+	-
Glucose-asparagine agar	+	-
Growth in 2% NaCl	-	+

Fig. 3. Phylogenetic tree showing the relationship among *Catenuloplanes*, *Couchioplanes*, and related bacteria.

Table 7. Differential characteristics of the genera belonging to the family *Micromonosporaceae*.

Genus	G+C content of DNA (mol%)	Aerial mycelium	Motility of arthrospore	Sporangium formation	Cell wall type	Glycolyl test	Major menaquinone (MK)	Phospholipid type	Fatty acid type	Whole-cell sugar
<i>Micromonospora</i>	71-73	-	-	-	II	Glycolyl	10(H ₄), 10(H ₆) / 9(H ₄), 9(H ₆)	PII	3b	xyl, ara
<i>Pilimelia</i>	ND	-	-	+	II	Acetyl	9(H ₂), 9(H ₄)	PII	2b	xyl, ara
<i>Actinoplanes</i>	72-73	-	-	+	II	Glycolyl	9(H ₄), 9(H ₆)	PII	2c	xyl, ara, gal
<i>Dactylosporangium</i>	72-73	-	-	+	II	Glycolyl	9(H ₈), 9(H ₆)	PII	2d	xyl, ara
<i>Catenuloplanes</i>	71-73	+	+	-	VI	Glycolyl	9(H ₈), 10(H ₈)	PIII	2c	xyl
<i>Couchioplanes</i>	70-72	+	+	-	VI	Glycolyl	9(H ₄)	PII	2c	xyl, ara, gal
<i>Catellatospora</i>	71-72	-	-	-	II	Glycolyl	10(H ₈ , H ₆) / 9(H ₄)	PII	ND	xyl, ara
<i>Glycomyces</i>	71-73	+	-	-	II	Glycolyl	10(H ₂ , H ₄)	PI	ND	xyl, ara

strains belong to the genera *Actinoplanes*, *Dactylosporangium*, *Micromonospora*, and *Pilimelia* of the family *Micromonosporaceae* Krassil'nikov 1938 emend. Goodfellow *et al.* 1990³³). However, I believe *Couchioplanes* and *Catenuloplanes* should be placed in the family *Micromonosporaceae* on the basis of the results of the comparative analysis of 16S rRNA sequences. For taxonomy of actinomycetes at the levels of the genus and family, the cell wall type by Lechevalier and Lechevalier³⁴) was very important criteria. Phylogenetic analysis clarified that the family *Micromonosporaceae* has cell wall type II and VI. Thus, phylogenetic analysis has proved to be very useful for classification at the family level of actinomycetes.

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