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Emended descriptions of the genus *Micrococcus*, *Micrococcus luteus* (Cohn 1872) and *Micrococcus lylae* (Kloos *et al.* 1974)

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Nine yellow-pigmented, spherical bacterial strains isolated from a medieval wall painting (strain D7), from indoor air (strains 3, 6, 7, 13C2, 38, 83 and 118) and from an activated-sludge plant (strain Ballarat) were classified by a polyphasic approach. Analyses of the 16S rRNA gene sequences of three representatives (strains D7, 118 and Ballarat) indicated that they all belong to the genus *Micrococcus*. The three isolates shared the highest sequence similarities with *Micrococcus luteus* DSM 20030^T (97·9–98%), *Micrococcus antarcticus* AS 1.2372^T (97·9–98·3%) and *Micrococcus lylae* DSM 20315^T (97·5–97·9%). DNA–DNA reassociation studies clearly demonstrated that all nine isolates belong to the species *M. luteus*. However, neither their chemotaxonomic features nor their physiological and biochemical properties were consistent with those of *M. luteus* DSM 20030^T. In contrast to *M. luteus* DSM 20030^T, all isolates investigated possessed MK-8(H_2) as the major respiratory quinone, and strain Ballarat had an A4α peptidoglycan type. On the basis of analyses of their Fourier transform-infrared spectroscopy spectra, isolates D7, 3, 6, 7, 13C2, 38, 83 and 118 could be grouped into a single cluster separate from *M. luteus* DSM 20030^T, strain Ballarat and *M. lylae* DSM 20315^T. In addition, all these isolates could be distinguished from *M. luteus* DSM 20030^T by their ability to assimilate D-maltose, D-trehalose, DL-3-hydroxybutyrate, DL-lactate, pyruvate and L-histidine and to hydrolyse casein. Strains D7, 3, 6, 7, 13C2, 38, 83 and 118 differed from both *M. luteus* DSM 20030^T and strain Ballarat by their ability to assimilate acetate, L-phenylalanine, L-serine and phenylacetate. Furthermore, REP-PCR fingerprinting yielded one common band for these strains, whereas this band was not observed for *M. luteus* DSM 20030^T, strain Ballarat or *M. lylae* DSM 20315^T. On the basis of these data, the species *M. luteus* can be divided into three biovars that are distinguished by several chemotaxonomic and biochemical traits: biovar I, represented by *M. luteus* DSM 20030^T; biovar II, represented by strains D7 (= DSM 14234 = CCM 4959), 3, 6, 7, 13C2, 38, 83 and 118; and biovar III, represented by strain Ballarat (= DSM 14235 = CCM 4960). On the basis of the results generated in this study, emended descriptions of the genus *Micrococcus* and the species *M. luteus* and *M. lylae* are given.

Keywords: *Micrococcus luteus*, 16S rRNA gene sequence, chemotaxonomy, physiological/biochemical traits

Abbreviations: pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl; FT-IR, Fourier transform-infrared spectroscopy.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of the *Micrococcus luteus* strains D7, 118 and Ballarat are respectively AJ409095, AJ312751 and AJ409096.

INTRODUCTION

The genus *Micrococcus* was first described by Cohn (1872). The description of the genus has been revised several times. Baird-Parker (1965) divided the aerobic, catalase-positive, Gram-positive cocci into two groups. Strains fermenting glucose were placed into group 1 and were later described as members of the genus *Staphylococcus*. Those utilizing glucose oxidatively, or not at all, were placed in group 2 (the genus *Micrococcus*). Rosypal *et al*. (1966) proposed a classification into groups based on the G+C content of the genomic DNA. Strains with a G+C content within the range 30·7–36·4 mol% were classified in the genus *Staphylococcus*, whereas it was proposed that strains with a G+C content within the range 66·3–73·3 mol% belonged to the genus *Micrococcus*. Subsequently, the genus *Micrococcus* was extended to include the species *Micrococcus lylae*, *Micrococcus kristinae*, *Micrococcus nishinomiyaensis*, *Micrococcus sedentarius* and *Micrococcus halobius* (Onishi & Kamekura, 1972; Kloos *et al*., 1974; Kocur *et al*., 1975). Later, 16S rDNA sequence analysis and chemical studies led to the fragmentation of the genus *Micrococcus* and the proposal of four new genera – *Kocuria*, *Nesterenkonia*, *Kytococcus* and *Dermacococcus* – while only two species, *Micrococcus luteus* and *M. lylae*, were considered to represent the genus *Micrococcus* (Stackebrandt *et al*., 1995). Recently, a cold-adapted bacterium has also been shown to belong to the genus *Micrococcus*, and the name *Micrococcus antarcticus* was proposed for this micro-organism (Liu *et al*., 2000).

During the investigation of bacteria isolated from indoor air (Camuffo *et al*., 1999), a wall painting and an activated-sludge plant, some bacterial isolates were obtained that were shown, by 16S rDNA sequence analysis, to be affiliated phylogenetically with the species *M. luteus*, but which clearly differed from it in terms of certain chemotaxonomic features and biochemical properties. Nevertheless, we demonstrate here that these isolates in fact represent biovars of the species *M. luteus*.

METHODS

Bacterial strains and cultural conditions. Strain D7 was isolated from a medieval wall painting in the chapel of Herberstein Castle in Styria, Austria. The airborne isolates 3, 6, 7, 13C2, 38, 83 and 118 were collected in the 'Museo Correr' in Venice, Italy, by using the Biotest Hycon air sampler Reuter-Centrifugal-Sampler Plus (Camuffo *et al*., 1999). Strain Ballarat was isolated from an activated-sludge plant in Ballarat, a city in the state of Victoria, Australia. All isolates were subcultivated on PYES-agar plates [l^{-1}: 3 g peptone from casein, 3 g yeast extract, 2·3 g disodium succinate, 15 g agar (all from Merck), pH 7·2] and incubated at room temperature. The strains used for comparison were *M. luteus* DSM 20030^T and *M. lylae* DSM 20315^T.

Morphological, physiological and biochemical characterization. Cell morphology was determined using phase-contrast microscopy. Gram-staining and the KOH test were performed as described previously (Moaledj, 1986). Catalase activity was detected by dropping 3% H_2O_2 onto well-developed colonies on agar plates and observing any gas bubbles produced. Oxidase activity was tested using Bactident Oxidase strips (Merck) according to the instructions of the manufacturer, and by using the method of Faller & Schleifer (1981). Carbon-source utilization tests and qualitative enzyme assays were carried out in microtitre plates as described previously (Kämpfer *et al*., 1991). Nitrate reduction, urease activity, the production of indole and hydrogen sulfide, the metabolism of citrate as well as aerobic acid production from carbohydrates and the hydrolysis of casein, starch, gelatin, Tween 20 and Tween 80 were tested as described by Smibert & Krieg (1994). pH tolerance was tested from pH 6 to pH 10 on buffered media (Nielson *et al*., 1995) and NaCl tolerance was measured at concentrations between 1 and 10% (w/v).

16S rDNA sequence analysis. The 16S rRNA genes from isolates D7, 118 and strain Ballarat were amplified, purified and sequenced as described by Wieser *et al*. (1999) and Maszenan *et al*. (1997). The sequences derived were aligned and compared with those of other bacterial 16S rDNA sequences available in the EMBL database by using the GCG FASTA program.

Nucleotide sequence accession numbers. The accession numbers for the reference sequences used are as follows: M38242 (*M. luteus* DSM 20030^T), X80750 (*M. lylae* DSM 20315^T) and AJ005932 (*M. antarcticus* AS 1.2372^T).

DNA isolation and characterization. Isolation of DNA from acetone-pre-washed biomass and DNA–DNA hybridization were performed as described previously (Wieser *et al*., 1999). REP-PCR was performed as described by Louws *et al*. (1994). The G+C content of DNA (mol%) was determined by using HPLC, according to Kaneko *et al*. (1986).

Chemotaxonomic investigations. Menaquinones, polar lipids and diagnostic cell-wall diamino acids were extracted and analysed as described previously (Wieser *et al*., 1999). Cellular fatty acid methyl esters were analysed according to Huys *et al*. (1997). Analysis of the peptidoglycan type was done as described by Groth *et al*. (1996).

Fourier transform-infrared (FT-IR) spectroscopy. Bacterial films for FT-IR spectroscopy were prepared from strains D7, 3, 6, 7, 38, 83, 118 and strain Ballarat, as well as from the type strains of *M. luteus* and *M. lylae*, grown on tryptic soy agar (Oxoid) at 28 °C for 48 h, by resuspending one loopful of cells in 80 µl deionized water and applying an aliquot of 35 µl to a predefined sample area on a zinc selenite optical plate. The samples were dried under reduced pressure to give a homogeneous film. The IR spectra were recorded in transmission mode between wave numbers 4000 and 500 cm^{-1} in an FT-IR spectrometer type IFS 28/B (Bruker), as described by Helm *et al*. (1991a, b). Data acquisition and analysis were carried out using the OPUS 3.0 software for bacterial identification from the same manufacturer. Mean spectra were calculated from six to nine independent measurements of *M. luteus* DSM 20030^T, *M. lylae* DSM 20315^T, strain Ballarat and strain 38, and from four to five independent measurements of the remaining strains. First derivatives of the spectra were compared with the hierarchical cluster-analysis methods implemented in OPUS 3.0; the spectral frequency ranges compared were nearly complete (4000–700 cm^{-1}) or were combinations of spectral windows W1–W5 (Helm *et al*., 1991a).

RESULTS AND DISCUSSION

Morphological and cultural characteristics

All of the strains that we studied were Gram-positive, catalase- and oxidase-positive cocci, mostly arranged in tetrads. They grew in circular, entire, convex and creamy yellow-pigmented colonies having diameters of approximately 4 mm after 2–3 days on PYES-agar plates at 37 °C.

Physiological and biochemical characteristics

The differentiating physiological and biochemical properties of isolates D7, 3, 6, 7, 13C2, 38, 83, 118 and Ballarat, as well as those of *M. luteus* DSM 20030^T and *M. lylae* DSM 20315^T, are summarized in Table 1. In contrast to *M. luteus* DSM 20030^T, all of the isolates investigated assimilated D-maltose, D-trehalose, DL-3-hydroxybutyrate, DL-lactate, pyruvate and L-histidine and hydrolysed casein. Isolates D7, 3, 6, 7, 13C2, 38,

Table 1. Physiological and biochemical properties that differentiate *M. antarcticus* AS1.2372^T, *M. luteus* DSM 20030^T, *M. lylae* DSM 20315^T and the novel isolates

+, Positive; −, negative; (+), weakly positive; Y, yellow; W, white; ND, data not available. Data for *M. antarcticus* are from Lui *et al.* (2000).

Property	*M. antarcticus* AS1.2372^T	*M. lylae* DSM 20315^T	*M. luteus* DSM 20030^T	D7	3	6	7	13C2	38	83	118	Ballarat
Pigmentation	Y	W	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Urease	−	−	+	+	−	−	+	−	−	−	−	+
Growth at pH 6	ND	−	−	+	−	(+)	+	(+)	(+)	+	(+)	−
Assimilation of:												
D-Mannose	−	−	+	+	+	+	+	+	+	+	+	+
D-Maltose	−	+	−	+	+	+	+	+	+	+	+	+
D-Trehalose	−	+	−	+	+	+	+	+	+	+	+	+
D-Xylose	−	−	−	−	−	−	+	−	−	−	−	−
Adonitol	ND	−	−	−	−	−	+	−	−	−	−	−
i-Inositol	ND	−	−	−	+	−	−	−	−	−	−	−
Maltitol	ND	+	+	+	+	−	+	+	+	+	+	−
D-Mannitol	ND	−	−	−	−	−	−	−	−	+	−	−
D-Sorbitol	−	−	−	−	−	−	−	−	−	+	−	−
Putrescine	ND	−	−	+	+	+	+	−	+	+	+	−
Acetate	−	+	−	+	+	+	+	+	+	+	+	−
Propionate	−	−	+	+	+	+	+	+	+	+	+	−
4-Aminobutyrate	ND	−	−	+	+	+	+	−	−	+	−	+
Citrate	ND	+	−	−	−	−	−	−	+	−	−	−
Fumarate	ND	+	−	+	+	−	+	+	+	+	+	+
Glutarate	ND	−	−	−	−	−	−	−	+	−	−	−
DL-3-Hydroxybutyrate	ND	+	−	+	+	+	+	+	+	+	+	+
DL-Lactate	ND	+	−	+	+	+	+	+	+	+	+	+
L-Malate	+	−	−	−	−	−	−	−	+	−	−	−
Oxoglutarate	ND	−	−	−	−	−	−	−	−	−	−	+
Pyruvate	−	+	−	+	+	+	+	+	+	+	+	+
L-Alanine	+	−	−	+	+	−	−	−	+	−	−	−
L-Aspartate	+	+	+	+	+	−	+	+	+	+	+	−
L-Histidine	ND	+	−	+	+	+	+	+	+	+	+	+
L-Leucine	ND	+	−	−	−	−	−	−	−	−	−	−
L-Phenylalanine	ND	−	−	+	+	+	+	+	+	+	+	−
L-Proline	ND	−	−	−	−	−	+	−	−	−	−	+
L-Serine	ND	−	−	+	+	+	+	+	+	+	+	−
3-Hydroxybenzoate	ND	+	−	−	−	−	−	−	−	−	−	−
4-Hydroxybenzoate	ND	+	−	−	−	−	−	−	−	−	−	−
Phenylacetate	ND	−	−	+	+	+	+	+	+	+	+	−
Hydrolysis of:												
L-Proline pNA	ND	+	+	+	+	+	+	+	+	+	+	−
Tween 20	+	+	+	+	+	+	−	+	+	+	+	+
Tween 80	+	+	−	−	−	−	−	−	+	+	−	−
Casein	ND	−	−	+	+	+	+	+	+	+	+	+

Table 2. Similarity matrix based on DNA–DNA hybridization data

Values are percentages of reassociation.

Strain	D7	Ballarat
D7	100	87·5
M. luteus DSM 20030^T	77·4	82·5
M. lylae DSM 20315^T	48·1	44·7
3	78·3	86·7
6	95·4	–
7	88·7	–
13C2	84·2	–
38	83·2	–
83	113·6	–
118	106·7	–
Ballarat	87·5	100

83 and 118 differed from both strain Ballarat and *M. luteus* DSM 20030^T in their ability to assimilate acetate, L-phenylalanine, L-serine and phenylacetate.

16S rDNA sequencing

The genus *Micrococcus* currently encompasses three species, *M. luteus*, *M. lylae* and *M. antarcticus*. These three are phylogenetically closely related to each other, although their status as separate species has been demonstrated by DNA relatedness (Schleifer *et al.*, 1979; Stackebrandt *et al.*, 1995; Liu *et al.*, 2000). It should be noted that 16S rDNA sequence similarity values obtained between these three species are higher than the value of 94·4 % reported by Liu *et al.* (2000). Analyses of the 16S rDNA of strains D7, 118 and Ballarat resulted in respective fragments of 1430 bases (positions 54–1505, *Escherichia coli* numbering; Brosius *et al.*, 1978), 1432 bases (53–1506) and 1424 bases (72–1507). The sequences of the three isolates shared the highest degrees of similarity to sequences of the type strains of *M. luteus* (97·9, 97·9 and 98 %), *M. lylae* (97·7, 97·5 and 97·9 %) and *M. antarcticus* (98·0, 97·9 and 98·3 %). Strain D7 exhibited a 16S rDNA sequence similarity of 99·3 % to strain 118 and of 99·0 % to strain Ballarat, which shared a sequence similarity of 98·9 % with strain 118. In agreement with this clustering, strains *M. luteus* DSM 20030^T, *M. lylae* DSM 20315^T, D7, 118 and Ballarat showed all the signature nucleotides specific to the family *Micrococcaceae* (Stackebrandt *et al.*, 1997), except those at positions 640, 839, 847 and 859 (*E. coli* numbering), where the nucleotides U, A, U and U were replaced by G, C, C and C, respectively.

DNA–DNA hybridization and G+C content of genomic DNA

DNA–DNA reassociation studies clearly revealed that all of the novel isolates investigated here belong to a single species whose members exhibit high levels of mutual relatedness (Table 2). Strains D7 and Ballarat had lower levels of DNA relatedness to *M. lylae* DSM 20315^T than to *M. luteus* DSM 20030^T. Thus, DNA–DNA reassociation studies performed with the type strains *M. luteus* DSM 20030^T and *M. lylae* DSM 20315^T and strains D7, 3, 6, 7, 13C2, 38, 83, 118 and Ballarat clearly support the view that all of these novel isolates belong to the species *M. luteus*, as they exhibit DNA relatedness values above 70 % (Table 2). The G+C contents of the genomic DNAs of strains D7 and Ballarat were determined to be 71 and 70 mol %, respectively, which match the G+C content of *M. luteus* perfectly (Stackebrandt *et al.*, 1995).

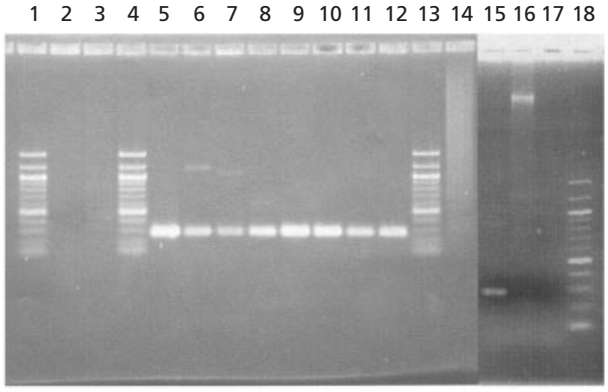

Fig. 1. DNA fingerprints obtained after using the REP-PCR technique from isolates D7 (lanes 5 and 15), 3 (lane 6), 6 (7), 7 (8), 13C2 (9), 38 (10), 83 (11), 118 (12) and Ballarat (16) and the reference strains *M. luteus* DSM 20030^T (lane 2) and *M. lylae* DSM 20315^T (3). Lanes 1, 4, 13 and 18, 100 bp ladder; lanes 14 and 17, negative control.

Analyses using the REP-PCR technique

REP-PCR yielded one common band for isolates D7, 3, 6, 7, 13C2, 38, 83 and 118, whereas no such band was obtained for *M. luteus* DSM 20030^T, *M. lylae* DSM 20315^T or strain Ballarat (Fig. 1). As the possibility exists that PCRs may be inhibited by cell-wall fragments or other cell substances, a second primer pair, corresponding to the 16S rDNA sequence, together with the REP primer set were subjected to PCR amplification. A PCR with these two primer sets generated two bands for strains D7, 3, 6, 7, 13C2, 38, 83 and 118 (the characteristic REP band at 300 bp and the 16S rRNA band at 1500 bp). Only the characteristic 16S rDNA band was observed for *M. luteus*, *M. lylae* and strain Ballarat (data not shown), demonstrating that PCR amplification with the DNA of these strains was not inhibited.

Chemotaxonomic features

The three species *M. luteus*, *M. lylae* and *M. antarcticus* have been described as differing in some chemotaxonomic properties, including their polar lipids, fatty acid profiles, quinone systems and cell-wall compositions. However, our data suggest that the differ-

Table 3. Fatty acid composition of *M. luteus* DSM 20030^T, *M. lylae* DSM 20315^T and the novel isolates

Values are percentages of total fatty acids. Abbreviations for fatty acids are illustrated by the following examples: $C_{16:0}$, hexadecanoic acid; i-$C_{15:0}$, 13-methyltetradecanoic acid; ai-$C_{15:0}$; 12-methyltetradecanoic acid. tr, Trace.

Fatty acid	*M. luteus* DSM 20030^T	*M. lylae* DSM 20315^T	D7	3	6	7	13C2	38	83	118	Ballarat
i-$C_{11:0}$	–	–	tr	tr	0·4	tr	0·6	tr	0·5	tr	–
ai-$C_{11:0}$	–	tr	tr	tr	tr	tr	1·0	0·5	0·6	0·8	–
i-$C_{13:0}$	tr	0·5	1·0	1·5	2·6	0·3	3·0	1·9	3·1	3·1	–
ai-$C_{13:0}$	tr	0·9	1·5	3·2	1·7	1·4	5·2	2·6	3·5	5·7	1·3
i-$C_{14:0}$	tr	2·7	2·2	3·7	2·1	2·4	2·8	2·0	3·1	3·3	1·2
$C_{14:0}$	3·7	0·8	tr	1·1	0·6	0·3	1·3	1·8	2·2	0·8	3·1
i-$C_{15:1}$	tr	–	tr	1·0	1·3	0·4	1·4	0·7	1·1	–	–
i-$C_{15:0}$	37·3	26·2	28·9	15·9	30·0	14·4	29·8	29·7	29·0	13·7	26·6
ai-$C_{15:0}$	52·5	59·3	63·9	70·1	58·4	78·0	53·2	56·7	51·8	70·7	61·0
i-$C_{16:1}$	tr	–	–	tr	tr	tr	tr	tr	0·6	–	–
i-$C_{16:0}$	tr	5·0	1·4	0·9	0·7	1·6	tr	1·1	1·1	0·9	1·4
$C_{16:0}$	2·5	0·9	tr	tr	tr	tr	tr	0·8	0·8	0·9	–
$C_{16:1}\omega 7c$	4·1	–	tr	1·1	0·8	tr	1·2	1·5	1·9	tr	–
i-$C_{17:0}$	tr	0·7	tr	tr	tr	tr	tr	tr	tr	tr	–
ai-$C_{17:0}$	tr	3·0	1·2	tr	0·6	1·2	tr	0·7	tr	0·7	–

ences in fatty acid profiles (Table 3) and polar lipid patterns of *M. luteus* DSM 20030^T and *M. lylae* DSM 20315^T are not as substantial as previously reported (Stackebrandt *et al.*, 1995).

The cellular fatty acid profiles of our isolates were of the iso and anteiso methyl-branched-chain type, with i-$C_{15:0}$ and ai-$C_{15:0}$ as the major components, accompanied by smaller, but significant, amounts of i-$C_{14:0}$ and i-$C_{16:0}$ (Table 3). The occurrence of straight-chain saturated fatty acids was reported to distinguish *M. luteus* DSM 20030^T from *M. lylae* DSM 200315^T when acids constituting $< 1\%$ of the total were not taken into consideration (Stackebrandt *et al.*, 1995). We have detected $C_{14:0}$ and $C_{16:0}$ fatty acids in the profile of *M. lylae* DSM 20315^T at levels of 0·8 and 0·9 %, respectively. It is well known that quantification of certain fatty acids is hard to reproduce at levels of 1% and below, and this may lead to the misinterpretation of data for classification purposes. For example, the fatty acid profiles of strains 3, 13C2, 38 and 83 were all similar to that of *M. luteus* DSM 20030^T, whereas strains D7, 6, 7 and 118 exhibited fatty acid profiles close to that obtained from *M. lylae* DSM 20315^T when amounts below 1% were ignored (Table 3).

Polar lipids also turned out to be unsuitable for differentiating *Micrococcus* species. In contrast to published data (Stackebrandt *et al.*, 1995), our results demonstrated unambiguously that *M. lylae* DSM 20315^T possesses small amounts of phosphatidylinositol in its polar lipid extract. All strains displayed identical polar lipid patterns, each possessing diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. Two unknown phospholipids and an unknown glycolipid were detected as well. Concentrations of phosphatidylinositol in strains D7, 3, 6, 7, 13C2, 38, 83, 118 and Ballarat appeared to be higher than that observed for *M. lylae* DSM 20315^T and lower than that detected for *M. luteus* DSM 20030^T.

Strains D7, 3, 6, 7, 13C2, 38, 83, 118 and Ballarat contained menaquinone MK-8(H_2) as the major quinone (64–84%). Significant amounts of MK-7(H_2) (8–25%) and MK-9(H_2) (3–12%) were also detected.

Cell-wall analysis was performed for strains D7, 3 and Ballarat, and, as expected, their diagnostic cell-wall diamino acid was L-lysine. However, strains D7 and 3 also contained alanine, glutamic acid and glycine. The molar ratio of these peptidoglycan amino acids (Ala : Glu : Gly : Lys) was 2·1 : 1·1 : 1·1 : 1·0 (strain D7). On the basis of amino acid and peptide analyses of cell-wall hydrolysates, strains D7 and 3 showed the peptidoglycan type of subgroup A2 according to Schleifer & Kandler (1972), with L-lysine, a peptide subunit as the interpeptide bridge and glycine bound to the α-carboxyl group of D-glutamic acid at position 2 of the peptide subunit. With strain Ballarat, the molar ratio of the peptidoglycan amino acids Ala : Glu : Gly : Lys : Asp was 1·9 : 1·0 : 1·6 : 0·2 : 0·5. The low contents of Asp and Lys were due to the occurrence of the peptide Asp → Lys, which is relatively stable under the conditions used for hydrolysis. In contrast to all other strains of *M. luteus* investigated, strain Ballarat possessed the peptidoglycan type A4α L-Lys–Asp with glycine bound to the α-carboxyl group of D-glutamic acid at position 2 of the peptide subunit (type A11.60 according to the 1998 DSMZ Catalogue of Strains), which has been found hitherto only in *Arthrobacter*

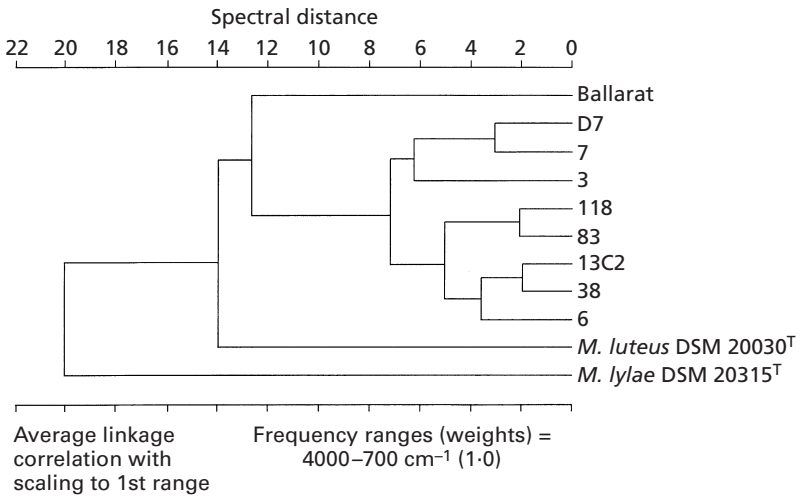

Fig. 2. Mean-linkage dendrogram based on distances of first derivatives of FT-IR spectroscopy spectra of *M. lylae* DSM 20315^T, *M. luteus* DSM 20030^T and strains Ballarat, D7, 7, 3, 118, 83, 13C2, 38 and 6. The dendrogram was constructed using the spectral window 4000–700 cm^{-1}.

woluwensis (Funke *et al.*, 1996). However, strain Ballarat also appears to belong to the species *M. luteus*, on the basis of DNA relatedness, even though the peptidoglycan type A11.60 has never been observed for members of the species *M. luteus*. It should be noted that the interpeptide bridge of *M. luteus* DSM 20030^T is structurally identical to the stem peptide in the peptidoglycan of strain Ballarat. In contrast, the stem peptides of *M. lylae* are linked via an aspartic acid residue. This suggests either that many strains of *M. luteus* are incapable of linking the stem peptides via a single amino acid (as is the case in *M. lylae* and strain Ballarat), but use a stem peptide to form the interpeptide bridge, or that the use of aspartic acid in the interpeptide bridge is an unusual feature of *M. lylae* and a few strains of *M. luteus*, since they cannot incorporate the peptide stem into the interpeptide bridge. It should be noted that the stem peptides of all *M. luteus* strains comprise alanine, glutamine, glycine and lysine, whereas glycine was absent from the cell walls of *M. lylae*. Published data on *M. antarcticus* also suggest that an A2-type cell wall, containing glycine, is present (Liu *et al.*, 2000).

FT-IR

Comparison of FT-IR spectra revealed a high degree of similarity between strains D7, 3, 6, 7, 13C2, 38, 83 and 118. When dendrograms based on various combinations of spectral windows were analysed, these strains always formed one cluster (not shown); in all but a few cases, distance values between these spectra were below 10, which might indicate that they are indistinguishable (Helm *et al.*, 1991a). Depending on the spectral window employed, the positions of strain Ballarat, *M. luteus* DSM 20030^T and *M. lylae* DSM 20315^T in the dendrograms relative to strains D7, 3, 6, 7, 13C2, 38, 83 and 118 did show some variation, *M. lylae* DSM 20315^T or *M. luteus* DSM 20030^T being more similar to the *Micrococcus* isolates investigated. This may not be significant, since the resolution of the FT-IR method may be at the intraspecific level (Tindall *et al.*, 2000). The grouping of the investigated *Micrococcus* strains into three clusters was in good agreement with the groupings produced by the chemotaxonomic data. However, agreement of the clustering with the groups obtained from the other results was obtained when the dendrogram was calculated on the basis of the spectral window between wave numbers 4000 and 700 cm^{-1} (Fig. 2). This observation may indicate that the window employed here is suitable for identifying strains of *M. luteus* by using FT-IR.

Considerations regarding heterogeneity in the species *M. luteus*

On the basis of the results presented here, members of the species *M. luteus* appear to possess quite diverse chemotaxonomic features with respect to their menaquinone systems, cell-wall compositions and FT-IR patterns, as well as biochemical properties. To our knowledge, no single bacterial species has been described that is characterized by such large differences in its quinone system and its peptidoglycan type. It should be noted that a survey of the literature indicates that members of a single species (when supported by DNA–DNA hybridization and phenotypic properties) usually provide us with a picture of a complex of strains highly similar in their phenotypic and genotypic properties. Such intraspecific differences as documented here have not been reported previously. However, despite the fact that our data may appear to contradict the widely accepted view of what constitutes a species, it should be pointed out that we do not know which mechanisms are responsible for the apparently significant variations in the quantitative menaquinone composition and qualitative peptidoglycan structure. It should also be pointed out that species tend to be regarded as static units, when they are, in fact, dynamic units that change over geological time. Normally, such changes are barely perceived by us, but examples of subdivisions within a single species, such as in the data presented here, may provide interesting evidence of a 'snapshot' of evolutionary change taking place.

Among the authors of this paper, there have been many discussions concerning how to treat the heterogeneity within the species *M. luteus*. One solution would have been to dissect the species *M. luteus* into three subspecies, but this would be premature. Whilst we wish to recognize the heterogeneity within the group, we felt that it would not be appropriate to give these groups subspecies status; instead, we elected to divide them, on a formal basis, into three different biovars. The recognition of three different biovars within the species *M. luteus* has the advantage that the three groups can be differentiated without nomenclatural changes having to be introduced. The results we have presented, and the conclusions we have drawn, indicate that a closer study of a larger number of isolates from the different species within the genus *Micrococcus* and from the different biovars within the species *M. luteus* may provide an interesting insight into the diversity within this group of organisms. On the basis of the data presented here, we propose the dissection of the species *M. luteus* into three biovars, as follows: biovar I, represented by *M. luteus* DSM 20030^T [peptidoglycan type A2, predominant quinones MK-8 and MK-8(H_2)]; biovar II, represented by strains D7, 3, 6, 7, 13C2, 38, 83 and 118 [peptidoglycan type A2, predominant quinone MK-8(H_2)]; and biovar III, represented by strain Ballarat [peptidoglycan type A4α, predominant quinone MK-8(H_2)]. Consequently, only selected physiological and biochemical properties appear to remain suitable for differentiating between *M. luteus* and *M. lylae* strains (Table 1).

On the basis of the results generated in this study, emended descriptions of the genus *Micrococcus* and the species *M. luteus* and *M. lylae* are presented.

Emended description of the genus *Micrococcus* Cohn 1872, 151^{AL}, emend. Stackebrandt *et al.* 1995, 691^{VP}

In addition to the properties given in the genus description (Stackebrandt *et al.*, 1995), members of the genus *Micrococcus* show several common characteristics. Growth occurs up to pH 10. The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, an unknown glycolipid and an unknown ninhydrin-negative phospholipid. L-Arabinose, *p*-arbutin, D-cellobiose, D-galactose, D-melibiose, D-ribose and salicin are not assimilated. Members of the genus share the *Micrococcaceae*-specific signature nucleotides at positions 293–304, 610, 598, 615– 625, 1025–1036, 1026–1035, 1265–1270 and 1278 of the 16S rRNA gene sequence (*E. coli* numbering) and lack the signature nucleotides at positions 640, 839–847 and 859 (Stackebrandt *et al.*, 1997).

Emended description of *Micrococcus luteus* (Schroeter) Cohn 1872, 153^{AL} (synonym: '*Bacteridium luteum*' Schroeter 1872, 126)

In addition to the properties given in the genus description above and the original species description, members of this species have the following characteristics. Growth or weak growth is observed at 45 °C, at pH 10 and in the presence of 10% NaCl; no growth is observed in the presence of 15% NaCl. Urease-variable. D-Glucose, sucrose and D-mannose are assimilated. D-Fructose, *N*-acetyl-D-glucosamine, L-rhamnose, gluconate, *cis*-aconitate, *trans*-aconitate, adipate, azelate, itaconate, mesaconate, suberate, β-alanine, L-ornithine, L-tryptophan, L-leucine, 3-hydroxybenzoate and 4-hydroxybenzoate are not assimilated. *p*-Nitrophenyl (pNP) α-glucopyranoside and L-alanine *p*-nitroanilide (pNA) are hydrolysed. pNP D-Galactopyranoside, pNP D-glucuronide, pNP D-glycopyranoside, bis-pNP phosphate, pNP phenylphosphate, pNP phosphorylcholine, 2-deoxythymidine-5′-pNP phosphate and L-glutamate-3-carboxy pNA are not hydrolysed. The peptidoglycan variation is either A2 or A4α. The predominant menaquinones are either MK-8 and MK-8(H_2) or MK-8(H_2) alone. MK-7 or MK-7(H_2) and MK-9(H_2) and MK-6(H_2) occur in minor amounts.

M. luteus biovar I

In addition to the properties given in the species description, members of this biovar have the following characteristics. No growth is observed at pH 6. Urease-positive. Maltitol, L-aspartate and propionate are assimilated. D-Maltose, D-trehalose, D-xylose, adonitol, *i*-inositol, D-mannitol, D-sorbitol, putrescine, acetate, 4-aminobutyrate, citrate, fumarate, glutarate, DL-3-hydroxybutyrate, DL-lactate, L-malate, oxoglutarate, pyruvate, L-alanine, L-histidine, L-phenylalanine, L-proline, L-serine and phenylacetate are not assimilated. L-Proline pNA and Tween 20 are hydrolysed. Casein and Tween 80 are not hydrolysed. The peptidoglycan type is A2. The major menaquinones are MK-8 and MK-8(H_2). The reference strain for this biovar is the designated type strain for this species, *M. luteus* DSM 20030^T.

M. luteus biovar II

In addition to the properties given in the species description, members of this biovar have the following characteristics. Colonies are creamy yellow in colour, circular, entire and convex. Growth at pH 6·0 is variable. Urease-variable. D-Maltose, D-trehalose, acetate, propionate, DL-3-hydroxybutyrate, DL-lactate, pyruvate, L-histidine, L-phenylalanine, L-serine and phenylacetate are assimilated. Oxoglutarate is not assimilated. Assimilation of D-xylose, adonitol, *i*-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, 4-aminobutyrate, citrate, fumarate, glutarate, L-malate, L-alanine, L-aspartate and L-proline is variable. Casein and L-proline pNA are hydrolysed. Hydrolysis of Tween 20 and Tween 80 is variable. The peptidoglycan type is A2. The major menaquinone is MK-8(H_2). The G+C content of the DNA of reference strain D7 is 71 mol%. Strains were isolated from a medieval wall painting and from indoor air. The reference strain for biovar II is strain D7 (= DSM 14234 = CCM 4959).

M. luteus biovar III

In addition to the properties given in the species description, the single member of this biovar has the following characteristics. Colonies are creamy yellow in colour, circular, entire and convex. No growth is observed at pH 6·0. Urease-positive. D-Maltose, D-trehalose, 4-aminobutyrate, fumarate, DL-3-hydroxybutyrate, DL-lactate, oxoglutarate, pyruvate, L-histidine and L-proline are assimilated. D-Xylose, adonitol, *i*-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, acetate, propionate, citrate, glutarate, L-malate, L-alanine, L-aspartate, L-phenylalanine, L-serine and phenylacetate are not assimilated. Tween 20 and casein are hydrolysed. L-Proline pNA and Tween 80 are not hydrolysed. The main menaquinone is MK-8(H_2). The peptidoglycan variation is A4α. The G+C content of the DNA of the reference strain is 70 mol%. Strain Ballarat was isolated from an activated-sludge plant in Ballarat, Victoria, Australia. The reference strain for biovar III is strain Ballarat (= DSM 14235 = CCM 4960).

Emended description of *Micrococcus lylae* (Kloos, Tornabene and Schleifer 1974, 83^{AL})

In addition to the properties given in the genus description above and the original species description, this species has the following characteristics. D-Glucose, sucrose, D-fructose, D-maltose, D-trehalose, maltitol, acetate, citrate, fumarate, DL-3-hydroxybutyrate, DL-lactate, pyruvate, L-aspartate, L-histidine, L-leucine, 3-hydroxybenzoate and 4-hydroxybenzoate are assimilated. D-Mannose, D-xylose, adonitol, *i*-inositol, D-mannitol, D-sorbitol, *N*-acetyl-D-glucosamine, L-rhamnose, gluconate, *cis*-aconitate, *trans*-aconitate, adipate, azelate, itaconate, mesaconate, suberate, putrescine, propionate, glutarate, L-malate, oxoglutarate, 4-aminobutyrate, L-ornithine, L-tryptophan, β-alanine, L-alanine, L-phenylalanine, L-proline, L-serine and phenylacetate are not assimilated. pNP α-Glucopyranoside, L-alanine pNA, L-proline pNA, Tween 20 and Tween 80 are hydrolysed. pNP D-Galactopyranoside, pNP D-glucuronide, pNP D-glycopyranoside, bis-pNP phosphate, pNP phenylphosphate, pNP phosphorylcholine, 2-deoxythymidine-5′-pNP phosphate, L-glutamate-3-carboxy pNA and casein are not hydrolysed. The main menaquinone is MK-8(H_2). The peptidoglycan variation is A4α.

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