Pseudochelatococcus lubricantis gen. nov., sp. nov. and Pseudochelatococcus contaminans sp. nov. from coolant lubricants

Peter Kämpfer,1 Stefanie P. Glaeser,1 Marco Gräber,2 Andreas Rabenstein,2 Jan Kuever2 and Hans-Jürgen Busse3

Correspondence
Peter Kämpfer
peter.kaempfer@umwelt.uni-giessen.de

1Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany
2Bremen Institute for Materials Testing, Department of Microbiology, Bremen, Germany
3Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria

Two Gram-negative, rod-shaped, non-spore-forming bacteria, isolated from metal working fluids were investigated to determine their taxonomic positions. On the basis of 16S rRNA gene sequence phylogeny, both strains (MPA 1113T and MPA 1105T) formed a distinct cluster with 97.7 % sequence similarity between them, which was in the vicinity of members of the genera Methylobacterium, Camelimonas, Chelatococcus, Bosea, Salinarimonas and Microvirga to which they showed low sequence similarities (below 94 %). The predominant compounds in the polyamine pattern and in the quinone system of the two strains were spermidine and ubiquinone Q-10, respectively. The polar lipid profiles were composed of the major compounds: phosphatidylmonomethylethanolamine, phosphatidyldiglycerol, phosphatidylcholine, major or moderate amounts of diphosphatidylglycerol, two unidentified glycolipids and three unidentified aminolipids. Several minor lipids were also detected. The major fatty acids were either C19:0 cyclo ω8c or C18:1 ω7c. The results of fatty acid analysis and physiological and biochemical tests allowed both, the genotypic and phenotypic differentiation of the isolates from each other, while the chemotaxonomic traits allowed them to be differentiated from the most closely related genera. In summary, low 16S rRNA gene sequence similarities and marked differences in polar lipid profiles, as well as in polyamine patterns, is suggestive of a novel genus for which the name Pseudochelatococcus gen. nov. is proposed. MPA 1113T (=CCM 8528T =LMG 28286T =CIP 110802T) and MPA 1105T (=CCM 8527T =LMG 28285T) are proposed to be the type strains representing two novel species within the novel genus, Pseudochelatococcus gen. nov., for which the names Pseudochelatococcus lubricantis sp. nov. and Pseudochelatococcus contaminans sp. nov. are suggested, respectively.

Metal working fluids (coolant lubricants) are used in machining processes such as turning, milling, drilling and grinding. Among the functions of these technical fluids is the cooling of pieces of work and tools, providing lubrication, and removal of chips and swarf from the work zone. Water miscible metal working fluids in the form of emulsions or solutions are commonly colonized by bacteria and fungi (Kämpfer et al., 2009a, b, c; Lodders & Kämpfer, 2012). Microbial degradation of components of the metalworking fluids causes a range of problems from the discoloration of the fluid and evolution of malodour to the loss of the quality of pieces of work and tool failure (Rabenstein et al., 2009). Moreover, high levels of bacterial and fungal contamination can lead to hygiene problems and pose a risk to the health of workers.

Two organisms, strains MPA 1105T and MPA 1113T, were isolated from metal working emulsions from tanks of machine tools at a factory in northern Germany using Plate Count agar (Merck KGaA,) and cultivation at 25 °C. Growth was also observed on Pseudomonas agar F (Merck KGaA,) and tryptone soy agar (TSA, Oxoid) at 25 °C.

DNA for 16S rRNA gene sequence analysis was extracted from pure cultures of both strains using a DNeasy Blood & Tissue kit (Qiagen). 16S rRNA gene fragments were amplified using the primers GM3F and GM4R as described...
previously (Kuever et al., 2001). Sequence analysis was carried out with the sequencing primers 515f and 907rm by LGC Genomics GmbH, Germany. A first BLAST analysis in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) showed that, using a cultivation independent approach to metal working fluid aerosols, several environmental 16S rRNA gene sequences had been detected, which were identical to the 16S rRNA gene sequence of strain MPA 1105\textsuperscript{T}, with one environmental 16S rRNA gene sequence identical to strain MPA 1113\textsuperscript{T} (Perkins & Angenent, 2010). BLAST analysis against the type strain database using the EzTaxon server (http://www.ezbiocloud.net/eztaxon; Kim et al., 2012), however, showed that the 16S rRNA gene sequence similarities with the most closely related type strains of the genus Chelatococcus were below 94\% for both strains.

Strains MPA 1113\textsuperscript{T} and MPA 1105\textsuperscript{T} formed beige-coloured colonies on nutrient agar (NA; Oxoid) at 37 °C. Subcultivation for further analyses was carried out on TSA at 28 °C for 48 h.

Gram-staining was performed as described by Gerhardt et al. (1994). Cell morphology was observed under a Axioskop 40 Zeiss light microscope at ×1000, with cells grown for 3 days at 28 °C on TSA.

Phylogenetic analysis was based on nearly full-length 16S rRNA gene sequences of 1406 and 1409 nt for MPA 1113\textsuperscript{T} and MPA 1105\textsuperscript{T} covering 16S rRNA gene sequence positions 24 to 1491 and 22 to 1491 [according to the E. coli numbering given by Brosius et al. (1978)], respectively. Analysis was performed in ARB release 5.2 (Ludwig et al., 2004) and the All-Species Living Tree' project (LTP; Yarza et al., 2008) database release LTPs115 (March 2014). SINA version 1.2.11 was used to align sequences not included in the database, according to SILVA seed alignment (http://www.arb-silva.de; Pruesse et al., 2012), before sequences were implemented into the ARB database. Phylogenetic trees were reconstructed with the Maximum-likelihood method using RAxML v7.04 (Stamatakis, 2006) with GTR-Gamma or PhyML (Guindon & Gascuel, 2003) and, the neighbour-joining method with the Jukes–Cantor correction (Jukes & Cantor, 1969) and rapid bootstrap analysis based on 100 resamplings. Phylogenetic trees were calculated based on nucleotide sequence positions 98 and 1449 of the 16S rRNA gene (E. coli numbering according to Brosius et al., 1978). Pairwise 16S rRNA gene sequence similarities were calculated with the ARB Neighbour joining tool without the use of an evolutionary model. The two new strains MPA 1113\textsuperscript{T} and MPA 1105\textsuperscript{T} shared 97.7\% sequence similarity to each other, but below 94\% sequence similarities to type strains of closely related genera, Chelatococcus (92.7–93.8\%), Camelimonas (91.5–92.8\%) and Bosea (91.5–92.7\%). Similarities to type strains of other genera were below 92\%. All phylogenetic trees showed that the two strains formed a distinct cluster, which changed position in the phylogenetic trees depending on the treeing method and algorithm used. In the maximum-likelihood tree, based on RAxML MPA 1113\textsuperscript{T} and MPA 1105\textsuperscript{T}, they were positioned closest to type strains of the genera Chelatococcus and Camelimonas (Fig. 1). In contrast, in the PhyML based maximum-likelihood tree the cluster of the two strains was placed between clusters of the genera Methylobacterium and Bosea and in the Neighbour-joining tree between clusters of the genera Methylobacter, Microvirga and Salinarimonas (data not shown). The unclear phylogenetic placement of the cluster containing the two new strains among clusters of different genera belonging to different families of the Alphaproteobacteria, indicated that there was a problem in clearly assigning the two strains to a family.

For quinone, polar lipid and polyamine analyses cells were grown on PYE medium (0.3\% w/v peptone from casein, 0.3\% w/v yeast extract, pH 7.2) at 28 °C. Polar lipids and quinones were extracted and analysed as described previously (Tindall, 1990a, b; Altenburger et al., 1996). Polymamines were extracted and analysed as described by Busse & Auling (1988). HPLC analyses were carried out using the equipment described by Stolz et al. (2007). The polar lipid profiles of strains MPA 1113\textsuperscript{T} and MPA 1105\textsuperscript{T} were similar, but showed a quite rare profile. Strain MPA 1113\textsuperscript{T} contained the major lipids phosphatidylmonomethylethanolamine, diphasphatidylglycerol, phosphatidylglycerol and phosphatidylcholine, moderate amounts of two glycolipids (GL1, GL2) and an aminolipid (AL3), and minor amounts of four aminolipids (AL1, AL2, AL4, AL5), and five lipids that were only visible after total polar lipid staining (L1–L5) (Fig. 2a). Strain MPA 1105\textsuperscript{T} differed in showing a polar lipid profile where the glycolipids GL1 and GL2, aminolipids AL1, AL2, AL3 and AL4 were also detected in major amounts, with the presence of another aminolipid (AL6) in moderate amounts and diphosphatidylglycerol present in minor amounts (Fig. 2b). The quinone system of strains MPA 1113\textsuperscript{T} and MPA 1105\textsuperscript{T} contained Q-10 (both 98.0\%) as the predominant ubiquinone. In addition, strain MPA 1113\textsuperscript{T} contained Q-9 (1.3\%), Q-11 (0.7\%) and traces of Q-8, while strain MPA 1105\textsuperscript{T} contained Q-11 (1.2\%), Q-9 (0.7\%) and Q-8 (0.2\%). In the polyamine pattern in both strains spermidine was predominant. Strain MPA 1113\textsuperscript{T} contained per g dry weight: 58.1 \(\mu\)mol spermidine, 8.4 \(\mu\)mol putrescine, 0.6 \(\mu\)mol spermine, 0.5 \(\mu\)mol 1,3-diaminopropane, 0.3 \(\mu\)mol sym-homospermidine and traces of cadaverine (<0.1 \(\mu\)mol). Strain MPA 1105\textsuperscript{T} contained per g dry weight: 34.2 \(\mu\)mol spermidine, 2.1 \(\mu\)mol spermine, 1.5 \(\mu\)mol putrescine, 1.5 \(\mu\)mol 1,3-diaminopropane, 0.4 \(\mu\)mol sym-norspermidine, 0.1 \(\mu\)mol sym-homospermidine and traces of cadaverine (<0.1 \(\mu\)mol). These chemotaxonomic traits agree well with traits already reported for members of the class Alphaproteobacteria but differ to some extent from other related genera of the Alphaproteobacteria (Table 1).

Fatty acids analysis was performed as described by Kämpfer & Kroppenstedt (1996). Total cell hydrolysates were generated from biomass after growth on TS agar for 48 h at 28 °C. We assessed the growth of the two strains and observed the colony sizes at 12 h intervals before selecting the time points for generating biomass. Growth (and colony expansion) could be clearly observed after 48 h of incubation.
compared with 60 h and 72 h of incubation. Significant differences were found between the fatty acid profiles of the two strains. MPA 1113T contained C18:1ω7c (86 %) as the major fatty acid, followed by C19:0cycloω8c (4.9 %), summed feature 2 (comprising 3-OH C14:0/C16:0 Iso I, 4.8 %) and C16:0 (4.3 %). MPA 1105T contained C19:0cycloω8c (91.3 %) as the major fatty acid, followed by C18:1ω7c (4.7 %) and minor amounts of summed feature 2 (comprising 3-OH C14:0/C16:0 Iso I, 2.0 %) and C16:0 (2.0 %). In contrast to the fatty acid profiles of those reported for species of the genera Camelimonas, Chelatococcus, Bosea, Salinarimonas and Microvirga, hydroxyl fatty acids were absent in the profiles of MPA 1113T and MPA 1105T (Table 1).

Detailed physiological characterization of strains MPA 1113T and MPA 1105T was performed as described previously (Kämpfer et al., 1991). The presence of urease was determined as recommended by the manufacturer (Merck), according to Christensen (1946). Results of the physiological characterizations are given in the species description. The two strains could be differentiated from their utilization of a few of the carbon substrates tested. D-Fructose (weakly), adipate (weakly), azelate, DL-3-hydroxybutyrate, itaconate, suberate, L-alanine, β-alanine, D-histidine, L-aspartate and L-proline, could only be assimilated by MPA 1113T. Furthermore, MPA 1113T grew in the presence of 1–5 % (w/v) NaCl, but strain MPA 1105T only in the presence of 1–3 % (w/v) NaCl.

For further genotypic characterization, high molecular mass genomic DNA was extracted using the method described by Pitcher et al. (1989). The genomic DNA G+C mol% content of strains MPA 1113T and MPA 1105T was

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**Fig. 1.** Maximum-likelihood tree based on nearly full-length 16S rRNA gene sequences showing the phylogenetic positions of strains MPA 1113T and MPA 1105T among type strains of the closest related genera. Numbers at branch notes represent Bootstrap values (>70 %) based on 100 resamplings. Numbers in cluster-boxes represent the number of type strains included in the clusters. *Aminobacter aminovorans* DSM 7048T, *Mesorhizobium loti* LMG 6125T, and *Phyllobacterium myrsinacearum* STM 948T were used as outgroups. Bar, 10 % divergence.
determined as described previously (Urdiain et al., 2008) and was \(58.2 (\pm 0.5)\) and \(53.3 (\pm 0.1)\) mol% for MPA 1113\(^T\) and MPA 1105\(^T\), respectively.

DNA–DNA hybridization experiments were performed between the two strains, MPA 1113\(^T\) and MPA 1105\(^T\), using the method described by Ziemke et al. (1998). The hybridization results gave a value of 49.2 % (37.1 %) (results of reciprocal analysis in parentheses), indicating that the two strains are representatives of two different species.

On the basis of the polyphasic approach we propose a novel genus with the name *Pseudochelatococcus* gen. nov. with two novel species, *Pseudochelatococcus lubricantis* sp. nov. and *Pseudochelatococcus contaminans* sp. nov., with type strains MPA 1113\(^T\) and MPA 1105\(^T\), respectively.

**Description of Pseudochelatococcus gen. nov.**

*Pseudochelatococcus* (Pseu.do.chel.at.o.cus. Gr. adj. pseudēs false; Chelatococcus a genus name; N.L. masc. n. Pseudochelatococcus the false Chelatococcus).

Cells are non-motile, non-spore-forming rods (approx. 2 \(\mu\)m in length and 1 \(\mu\)m in width). Gram-stain-negative, oxidase-positive, showing an oxidative metabolism. Good growth occurs on R2A, TSA, PYE, and nutrient agar at 25–30 °C. Beige, translucent and shiny colonies with entire edges are formed within 24 h, with a diameter of approximately 2 mm. Predominant compound in the polyamine pattern is spermidine. The quinone system is ubiquinone Q-10. The polar lipid profile is composed of the major lipids phosphatidylmonomethylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylcholine and moderate to major amounts of two glycolipids. Major fatty acids are \(C_{19:0}\) cycl\(\omega8c\) and/or \(C_{18:1}\)\(\omega7c\).

The \(G+C\) content of the DNA is different for different species of the genus, \(58.2 (\pm 0.5)\) (type species) and \(53.3 (\pm 0.1)\) mol%. The type species is *Pseudochelatococcus lubricantis*.

**Description of Pseudochelatococcus lubricantis** sp. nov.

*Pseudochelatococcus lubricantis* [lubri.can’tis. L. v. lubricare to lubricate; N.L. n. lubricans, -antis (from L. part. adj. lubricans) a lubricant; N.L. gen. lubricantis of/from a (coolant) lubricant].

Cell and colony morphology and growth characteristics are as given in the genus description. Growth is detected on TSA at temperatures ranging from 4 °C to 50 °C (weak). No growth is observed at 0 °C and 55 °C. Growth is also positive in TS broth at pH values ranging from 5.5–10.5. Growth is observed in TS broth containing (in addition) 1–5 % (w/v) NaCl. Beige, translucent and shiny colonies with entire edges are formed within 24 h, with a diameter of approximately 2 mm. The quinone system and polyamine pattern are in agreement with the genus description. In addition to the lipids listed in the genus description moderate amounts of an aminolipid (AL3) and minor amounts of four amino-lipids (AL1, AL2, AL4, AL5) and five lipids only visible after total polar lipid staining (L1–L5) are present. The fatty acid profile is dominated by \(C_{19:0}\) cycl\(\omega8c\) and contains a minor amount of \(C_{18:1}\)\(\omega7c\). Utilizes the following carbon
Table 1. Traits distinguishing Pseudochelatococcus gen. nov. from the related genera Camelimonas, Chelatococcus, Methylobacterium, Bosea, Salinarimonas and Microvirga

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<td>Glycolipids, GL1 and GL2§</td>
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† Data from Auling et al. (1993), Yoon et al. (2008).
‡ Data from Busse & Auling (1988); Auling et al. (1991); Tani et al. (2012a, b); Weon et al. (2008); Veyisoglu et al. (2013).
§ Das et al. (1996).
∥ Liu et al. (2010).
¶ Weon et al. (2010); Ardley et al. (2012); Radl et al. (2014).
§§ For chromatographic motifs of glycolipids GL1 and GL2 see Fig. 2.

Sources: D-fructose (weakly), acetate, propionate, 4-amino- butyrate, adipate (weakly), azelate, fumarate, glutarate, DL-3- hydroxybutyrate, itaconate, DL-lactate, L-malate, pyruvate, suberate, L-alanine, β-alanine, D-histidine, L-aspartate and L-proline. Does not utilize D-glucose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-arabinose, D-galactose, gluconate, D-mannose, maltose, alpha-melibiose, L-ribose, salicin, sucrose, trehalose, D-xylene, adonitol, inositol, maltol, D-mannitol, D-sorbitol, cis-aconitate, trans-aconitate, mesaconate, oxoglutarate, putrescine, L-ornithine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate. Hydrolyses: L-alanine-pNA (pNA = para-nitroanilide) and 2-deoxymyristinolide-5′-pNP-phosphate and does not hydrolyse: aesculin, oNP-β-D-galactopyranoside (oNP=ortho-nitrophenyl-), pNP-β-D-glucuronide (pNP=para-nitrophenyl-), pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, pNP-β-D-xylidine, and L-glutamate-gamma-3-carboxy-pNA.

The type strain MPA 1105T (=CCM 8527T=LMG 28285T) was isolated from coolant lubricants. The G+C content of the genomic DNA of the type strain is 58.2 mol% (+/−0.5).

Description of Pseudochelatococcus contaminans sp. nov.

Pseudochelatococcus contaminans [con.ta’mi.nans. L. part. adj. contaminans contaminating, polluting, isolated as a contaminant of an industrial coolant lubricant).

Cell and colony morphology and growth characteristics are as given in the genus description. Growth is detected on TSA at temperatures ranging from 4 °C to 50 °C (weak). No growth is observed at 0 °C and 55 °C. Growth is also positive in TS broth at pH values ranging from 5.5–10.5. Growth is observed in TS broth containing (in addition) 1–3% (w/v) NaCl. Beige, translucent and shiny colonies with entire edges are formed within 24 h, with a diameter of approximately 2 mm. The quinone system and polyamine pattern are in agreement with the genus description. In addition to the quinones listed in the genus description, menaquinones MK7 and MK8 and ortho-para-nitrophenyl-quinones are detected in major amounts; another quinone (Q8) is detected in moderate amounts and diphasitadglycerol is present in minor amounts. The fatty acid profile is predominated by C19:0 cyclo ω8c and contains minor amounts of C18:1ω7c. Utilizes the following carbon sources: acetate, propionate, 4-amino- butyrate, fumarate, glutarate, DL-lactate, L-malate and pyruvate. Does not utilize D-glucose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-arabinose, D-fructose, D-galactose, gluconate, D-mannose, maltose, alpha-melibiose, L-ribose, salicin, sucrose, trehalose, D-xylene, adonitol, inositol, maltol, D-mannitol, D-sorbitol, cis-aconitate, trans-aconitate, adipate, azelate, DL-3-hydroxybutyrate, itaconate, mesacone, oxoglutarate, putrescine, suberate, L-alanine, β-alanine, D-histidine, L-aspartate, L-proline, L-ornithine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate. Hydrolyses: L-alanine-pNA (pNA = para-nitroanilide) and 2-deoxymyristinolide-5′-pNP-phosphate (weakly). Does not hydrolyse: aesculin, oNP-β-D-galactopyranoside (oNP=ortho-nitrophenyl-), pNP-β-D-glucuronide (pNP=para-nitrophenyl-), pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, and L-glutamate-gamma-3-carboxy-pNA.

The type strain MPA 1105T (=CCM 8527T=LMG 28285T) was isolated from coolant lubricants. The G+C content of the genomic DNA of the type strain is 53.3 mol% (+/−0.1).

References


