Rubellimicrobium thermophilum gen. nov., sp. nov., a red-pigmented, moderately thermophilic bacterium isolated from coloured slime deposits in paper machines

Ewald B. M. Denner,^{1,2†} Marko Kolari,² Douwe Hoornstra,² Irina Tsitko,² Peter Kämpfer,³ Hans-Jürgen Busse⁴ and Mirja Salkinoja-Salonen²

Correspondence

Ewald B. M. Denner
ewald.denner@vu-wien.ac.at

¹Institut für Mikrobiologie und Genetik, Universität Wien, A-1030 Wien, Austria

²Department of Applied Chemistry and Microbiology, POB 56, FIN 00014 University of Helsinki, Finland

³Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35390 Giessen, Germany

⁴Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria

Six red-pigmented strains of the *Alphaproteobacteria* with optimal growth between 45 and 54 °C were previously isolated from coloured biofilms in two fine-paper machines and one pulp dryer. The strains were found to be resistant to 15 p.p.m. 2,2-dibromo-3-nitropropionamide, a common industrial biocide. 16S RNA gene sequence similarity of the isolates was 99.7–100 %. Ribotyping using the restriction enzymes *PvuII* and *EcoRI* showed that four of the isolates (C-lvk-R2A-1, C-lvk-R2A-2^T, C-R2A-52d and C-R2A-5d) belong to a single species. 16S rRNA gene-based phylogenetic analysis revealed that, together with *Rhodobacter blasticus* ATCC 33485^T, the isolates form a deep line of descent (94.7–94.9 % sequence similarity) within the family *Rhodobacteraceae* loosely affiliated with the *Rhodobacter/Paracoccus* clade. The isolates were strictly aerobic and oxidase-positive (catalase was weakly positive) and utilized a wide range of substrates including pentoses, hexoses, oligosaccharides and sugar alcohols. The predominant constituents in their cellular fatty acid profiles were C_{19:0} cyclo ω 8c (39–44 %), C_{18:0} (21–24 %) and C_{16:0} (21–23 %). Fatty acids present in smaller amounts included C_{18:1} ω 7c, C_{10:0} 3-OH, C_{18:1} ω 7c 11-methyl, C_{20:2} ω 6,9c and C_{17:0} cyclo, amongst others. Polar lipids included diphosphatidylglycerol, phosphatidylcholine and an unidentified aminolipid, but not phosphatidylethanolamine. Carotenoid pigments were synthesized but bacteriochlorophyll *a* was not. The polyamine patterns consisted of the major compounds putrescine, spermidine and sym-homospermidine. The major respiratory lipoquinone was ubiquinone Q-10. The DNA G + C content was 69.4–70.2 mol%. On the basis of the phylogenetic and phenotypic evidence, the biofilm isolates were classified in a new genus, *Rubellimicrobium* gen. nov.; four of the isolates are assigned to the type species, *Rubellimicrobium thermophilum* gen. nov., sp. nov. Strain C-lvk-R2A-2^T (= CCUG 51817^T = DSM 16684^T = HAMBI 2421^T) is the type strain of *Rubellimicrobium thermophilum*.

Coloured microbial slime deposits (biofilms) in the wet end of paper machines are deleterious, particularly for white end products. Bacterial slime deposits on machine surfaces

impair the runability of paper machines, because thick biofilms may break loose and cause web breaks or product defects such as holes and coloured spots (Blanco *et al.*,

[†]Present address: Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria.

Abbreviations: HSPD, sym-homospermidine; pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl; PUT, putrescine; SPD, spermidine.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains A-col-R2A-4, C-lvk-R2A-2^T, C-R2A-52d, E-R2A-8a and C-lvk-R2A-1 are AJ505840 and AJ844281–AJ844284, respectively.

Details of the polyamine and fatty acid profiles of the novel strains, characteristics that distinguish the strains from other similar species isolated from paper-machine biofilms, results of riboprint analysis and additional electron micrographs are available as supplementary material in IJSEM Online.

1996). Knowledge of the microbial diversity in a pulp and paper mill therefore provides a rational basis for the development of effective control strategies. Consequently, numerous studies over the past decade have been dedicated to bacteria in paper-machine water and slimes and a wide range of species has been isolated (Väisänen *et al.*, 1994, 1998; Chaudhary *et al.*, 1997; Pellegrin *et al.*, 1999; Kolari *et al.*, 2001; Oppong *et al.*, 2000; Desjardins & Beaulieu, 2003; Suihko *et al.*, 2004; Rättö *et al.*, 2005).

Recently, Kolari *et al.* (2003) performed a survey of coloured biofilms from six different paper machines, including two case studies of outbreaks of coloured slimes in which the causative bacteria were found. The bacteria were isolated in the years 1999–2001 from biofilms scraped off from fine-paper machine surfaces and from a pulp dryer. Four groups of pink- or red-pigmented bacteria that were isolated from several mills were identified. *Deinococcus geothermalis* (group I) and *Meiothermus silvanus* (group II) were found as common primary biofilm-formers in paper machines. Group III contained *Roseomonas*-like strains that were not biofilm formers, but which were common in slimes of neutral or alkaline machines. Group IV consisted of strains representing a novel genus related to *Rhodobacter*. The 'group IV' isolates ($n = 6$) were obtained from coloured slime deposits in two paper machines and a pulp dryer in different seasons, suggesting that these bacteria are regular inhabitants of coloured paper-mill slimes. The isolates formed thick biofilms on stainless-steel test coupons exposed to neutral paper-machine process water and resisted the common industrial biocide 2,2-dibromo-3-nitrilopropionamide at 15 p.p.m. In this study, we report on the polyphasic taxonomic characterization of these isolates.

16S rRNA gene-based phylogeny, riboprint patterns and base composition of DNA

Direct sequencing of PCR-amplified 16S rRNA gene sequences was carried out as described by Rainey *et al.* (1996). The six isolates showed >99% 16S rRNA gene sequence similarity, indicating a high degree of relatedness. By riboprint analysis (see Supplementary Fig. S1 available in IJSEM Online), isolates C-lvk-R2A-1, C-lvk-R2A-2^T and C-R2A-5d were almost indistinguishable; isolate C-R2A-52d showed minor differences. According to UPGMA clustering with the Dice coefficient, the riboprints of isolates C-lvk-R2A-1, C-lvk-R2A-2^T, C-R2A-52d and C-R2A-5d showed >65% similarity. Isolates E-R2A-8a and A-col-R2A-4 showed only moderate (~50 and 40%) riboprint similarity (Supplementary Fig. S1). Ribotyping clearly demonstrated that isolates C-lvk-R2A-1, C-lvk-R2A-2^T, C-R2A-52d and C-R2A-5d belong to a single species. Isolates E-R2A-8a and A-col-R2A-4 may represent separate species. Sequence similarity searching against nucleotide databases using the fast3 program (Pearson & Lipman, 1988; Pearson, 1990) indicated that the 'group IV' isolates are most closely related to strains and species of the family *Rhodobacteraceae* (order *Rhodobacterales*) of the class *Alpha-proteobacteria* (Garriety *et al.*, 2005, 2006). The highest 16S

rRNA gene sequence similarities were found to *Rhodobacter blasticus* ATCC 33485^T (94.7–94.9%) and *Paracoccus kocurii* JCM 7684^T (93.8–94%). This finding is in good agreement with previous data (Kolari *et al.*, 2003). Dendrograms of phylogenetic relationships inferred from neighbour-joining (Fig. 1) and both maximum-likelihood and maximum-parsimony analysis (data not shown) including a subset of *Rhodobacteraceae* showed that the bacteria represent a separate subline of descent that is loosely affiliated with the *Rhodobacter* supercluster of organisms, sharing a branching point with *Rba. blasticus* ATCC 33485^T. Bootstrap analysis gave 100% confidence for this branching.

The DNA base compositions, analysed by HPLC (Cashion *et al.*, 1977; Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984), of isolates C-R2A-lvk-2^T, C-R2A-5d, E-R2A-8a and A-col-R2A-4 were 70.0, 70.2, 69.9 and 69.4 mol% G+C, respectively. These G+C contents do not differ significantly from those of other species in this group.

Chemotaxonomic characteristics

Respiratory lipoquinone analysis by HPLC (Tindall, 1990; Altenburger *et al.*, 1996) showed that the 'group IV' isolates possess ubiquinones exclusively, with Q-10 predominant (84–100%); minor amounts of Q-9 were also present. The pattern of cellular polyamines (Supplementary Table S1) analysed as described by Busse & Auling (1988) and Busse *et al.* (1997) was characterized by the major compounds *sym*-homospermidine (HSPD), spermidine (SPD) and putrescine (PUT). This polyamine pattern is not in agreement with those reported for other members of the *Rhodobacteraceae*. Species in this group investigated so far, including members of *Paracoccus*, *Rhodobacter*, *Rhodovulum* and *Roseomonas*, contain SPD and PUT or only SPD as major components; HSPD is absent (Busse & Auling, 1988; Hamana & Takeuchi, 1998). Polyamine data for other taxa in this group are not available. Thus, the presence of HSPD may indicate a separate phylogenetic position, if it is not an adaptation to higher temperatures. In this context it would be interesting to know whether the closest relative *Rba. blasticus* shares this trait; a similar polyamine pattern would indicate that the 'group IV' isolates and *Rba. blasticus* should not be considered as members of the *Rhodobacteraceae*.

The polyamine patterns of isolates E-R2A-8a and A-col-R2A-4 differed from those of the other four isolates (Supplementary Table S1). Isolate E-R2A-8a showed significantly higher ratios of SPD:PUT (2.3) and SPD:HSPD (2.0). Isolate A-col-R2A-4 differed from the other isolates in a threefold higher polyamine content. The differences in the polyamine patterns indicate some quantitative variability within the isolates.

The fatty acid profiles of the 'group IV' isolates analysed by GLC as described by Väisänen *et al.* (1994) were almost identical (Supplementary Table S2). The predominant components were C_{19:0} cyclo ω 8c (39–44%), C_{18:0}

Fig. 1. Dendrogram based on 16S rRNA gene sequence data, indicating the phylogenetic position of the 'group IV' isolates. 16S rRNA gene sequences were retrieved from the EMBL or RDP databases (Stoesser *et al.*, 2002; Cole *et al.*, 2003), aligned manually using the program PILEUP (Devereux *et al.*, 1984) and edited to remove ambiguous nucleotide positions and alignment gaps. A continuous stretch of 1099 nucleotides in the alignment was used in pairwise evolutionary distance estimation. For construction of the phylogenetic dendrogram, operations included in the PHYLIP software package (Felsenstein, 1995) were used. Pairwise evolutionary distances were computed from percentage similarities by the correction of Jukes & Cantor (1969). Based on the evolutionary distance values, the phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987). Bar, 10 % sequence divergence.

(21–24 %) and C_{16:0} (21–23 %). This fatty acid signature differed from those of the majority of species in the order *Rhodobacterales* mainly in the proportions of C_{19:0} cyclo ω 8c and C_{18:1} ω 7c. Interestingly, the 'group IV' isolates produced *cis*-8-cyclo-nonadecanoic acid (C_{19:0} cyclo ω 8c) as the major (>35 %) fatty acid. Consulting published data, significant amounts of cyclopropane (C_{19:0}) acids are typical of species of the order *Rhizobiales* (Kuykendall, 2005) such as *Rhodovarius lipocyclicus* (Kämpfer *et al.*, 2004), *Marteella mediterranea* (Rivas *et al.*, 2005), *Ochrobactrum gallinifaecis* (Kämpfer *et al.*, 2003) and *Pleomorphomonas oryzae* (Xie & Yokota, 2005), some rhizobia (Tighe *et al.*, 2000) and of facultative serine-pathway methylobacteria

(Doronina *et al.*, 2000). Within the order *Rhodobacterales*, C_{19:0} cyclo ω 8c as a major component has been reported for *Jannaschia helgolandensis* and *Oceanicola batsensis* (Wagner-Döbler *et al.*, 2003; Cho & Giovannoni, 2004). A further characteristic of the 'group IV' isolates as determined by GC-MS analysis (data not shown) was the lack of 3-oxo tetradecanoic acid, which had been reported as a common characteristic of members of the *Rhodobacter/Paracoccus* group (Krauss *et al.*, 1989; Neumann *et al.*, 1995).

The polar lipid fingerprints obtained by two-dimensional TLC (Denner *et al.*, 2001) were identical, consisting of the

major compounds diphosphatidylglycerol, phosphatidylcholine and an unknown aminolipid. Additionally, phosphatidylglycerol was detected in minor amounts. The majority of these lipids have been detected in a multitude of taxa within the *Alphaproteobacteria*. Phosphatidylethanolamine, commonly present in Gram-negative bacteria, was not detected in lipid extracts of the 'group IV' isolates. Within the *Rhodobacteraceae*, the absence of phosphatidylethanolamine has also been reported for *Antarctobacter heliothermus*, *Roseobacter denitrificans*, *Roseobacter litoralis*, *Roseisalinus antarcticus* and *Thalassobacter stenotrophicus* (Labrenz *et al.*, 1998, 1999, 2005; Macián *et al.*, 2005).

Morphological, physiological and biochemical characteristics

For phenotypic characterization, all isolates were routinely cultivated on R2A agar (BBL) at 45 °C. Colony morphology was observed visually and recorded after 48 h growth. Standard bacteriological testing was performed as described by Smibert & Krieg (1994). Growth under anaerobic conditions was tested by incubating R2A agar plates in anaerobic jars using the AnaeroGen anaerobic atmosphere generation system (Oxoid). Maximum growth temperature ($T_{\max}$) was determined by incubating inoculated R2A agar plates in a Stuart scientific biological incubator S101D (Bibby Sterilin) with gradual temperature increments in steps of 3 °C. Each isolate was grown for 2 days, growth was assessed and a new agar plate was inoculated prior to the next temperature increment. Incubator temperature was recorded (± 0.2 °C) by a Tinytag model G temperature datalogger (Gemini Data Loggers). Cell-wall type was determined by the Gram-staining method (Smibert & Krieg, 1994) and was confirmed by the KOH lysis test (Buck, 1982) and L-alanine aminopeptidase test (Merck). Metabolic profiling was made by means of a miniaturized assay (Kämpfer *et al.*, 1991). Antimicrobial susceptibility testing (disc diffusion method) was performed by using commercial antibiotic-impregnated discs (Oxoid). Briefly, 100 µl of a bacterial suspension (McFarland standard 0.5) was plated onto R2A agar and incubated for 48 h at 45 °C. Any zone of inhibition was scored as sensitivity to that antimicrobial compound. Light microscopic examinations were performed on a Leitz Diaplan microscope equipped with phase-contrast optics (magnification $\times 1000$). For transmission electron microscopy, cells were pre-fixed for 4 h at room temperature with 2.5 % (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) and washed four times in the same buffer. For negative staining, a drop of cell suspension was placed on a copper grid coated with Pioloform and carbon and stained with 1 % potassium phosphotungstate adjusted to pH 6.5 with potassium hydroxide. Thin-sectioned samples were post-fixed in buffered 1 % (w/v) osmium tetroxide for 2 h, dehydrated in a graded series of ethanol and acetone and embedded in Epon LX-112 resin. Thin sections were cut with a diamond knife on a Leica Ultracut UCT ultramicrotome and double-stained with uranyl acetate and lead citrate. Samples were

examined (operating voltage 60 kV) on a JEM-1200EX transmission electron microscope (JEOL).

Colonies of the 'group IV' isolates on R2A agar were translucent, smooth, circular, slightly convex and red-pigmented. Isolates A-col-R2A-4 and E-R2A-8a were less intensely pigmented. The absorption spectra of pigments recorded with a Hitachi S-2000 spectrophotometer from whole cells extracted with acetone/methanol (7:2, v/v) gave a major peak at 495 nm and two shoulders at 465 and 525 nm. Carotenoid tests with concentrated sulphuric acid and iodine/potassium iodide showed the characteristic colour changes to blue and green, respectively. Bacteriochlorophyll *a* was not detected under any experimental conditions. Cells of all 'group IV' isolates were rod-shaped ($\sim 0.6\text{--}0.8 \times 2.0\text{--}4.0$ µm), stained Gram-negative, lysed in the presence of 3 % (w/v) KOH and showed L-alanine aminopeptidase activity. Electron microscopic examinations of ultrathin sections indicated the presence of unusual non-prosthecae, curled cell-surface structures (Fig. 2). Cells were partially attached to self-secreted polymeric substances (Supplementary Figs S2 and S3). Intracellular inclusion bodies of polyphosphate and polyhydroxyalkanoates were found in the cytoplasm of the majority of cells. Whether the curled cell-surface structures play a role in biofilm formation remains to be determined.

Cultivation experiments showed that the isolates did not grow on nutrient-rich peptone-based media such as tryptone soy agar (Oxoid) or brain heart infusion agar (Difco). However, good growth was observed when these media were diluted tenfold. The optimum temperature for growth was between 45 and 54 °C; $T_{\max}$ was 56 °C. The isolates utilized a variety of compounds as sole sources of carbon and energy for growth, including hexoses, pentoses and sugar alcohols, but only a few amino acids and organic acids. Acid was not formed from any sugar compound tested. The metabolic profiles of the six isolates were identical. Further details on phenotypic characteristics are given in Table 1 and in the description of the novel taxon. Key diagnostic characteristics useful in the initial identification of aerobic pink- to red-pigmented bacteria from paper-machine biofilms are compiled in Supplementary Table S3.

Taxonomic considerations

Phylogenetic analyses based on 16S rRNA gene sequence data showed that the 'group IV' isolates represent a separate subline of descent within the family *Rhodobacteraceae*, with *Rba. blasticus* as the closest phylogenetic neighbour (Fig. 1). It is evident from riboprint analyses that four of the isolates (C-lvk-R2A-1, C-lvk-R2A-2^T, C-R2A-52d and C-R2A-5d) represent a single species (Supplementary Fig. S1). This is also supported by highly similar polyamine patterns and fatty acid profiles (Supplementary Tables S1 and S2). Isolate E-R2A-8a might be another strain of this species, because it shared the majority of characteristics with these strains. The moderate riboprint similarity of E-R2A-8a, however, did not allow its unambiguous assignment. Isolate A-Col-R2A-4

Fig. 2. Electron micrographs of ultrathin sections of isolate E-R2A-8a. (a) The Gram-negative cell-wall structure is visible, as are polyphosphate (black) and polyhydroxyalkanoate (white) inclusion bodies. Note the tiny curled structures on the cell surface. Bar, 500 nm. (b) Exceptional curl-like structures are visible on the cell surface. Bar, 100 nm.

also shared the majority of characteristics with the other five isolates, but there is some evidence that this isolate may represent a second species (isolate A-Col-R2A-4 had the lowest riboprint similarity to the other isolates). In addition, the polyamine content was approximately three-fold higher and the HSPD:PUT and HSPD:SPD ratios were significantly higher in isolate A-Col-R2A-4 than in the

other isolates (Supplementary Table S1). Further molecular genetic analyses will solve the taxonomic status of E-R2A-8a and A-Col-R2A-4. For the present, however, we propose the novel genus and species *Rubellimicrobium thermophilum* for isolates C-lvk-R2A-1, C-lvk-R2A-2^T, C-R2A-52d and C-R2A-5d. Isolates E-R2A-8a and A-Col-R2A-4 are tentatively classified as *Rubellimicrobium* sp.

Table 1. Key taxonomic characteristics of the genus *Rubellimicrobium* gen. nov. and related genera

+, Present or positive; –, absent or negative; V, varies between species; ND, no data available.

Characteristic	<i>Rubellimicrobium</i>	<i>Rhodobacter</i>	<i>Paracoccus</i>
Pigment production	+	+	V
Cell shape	Rods	Ovoid to rod-shaped	Coccoid to short rods
Intracytoplasmic membranes	–	+	–
Motility	+	V	–
Optimum growth temperature (°C)	45–54	30–35	25–42
Mode of respiration	Aerobic	Facultative	Aerobic
Bacteriochlorophyll <i>a</i>	–	+	–
Growth physiology	Chemoheterotroph	Chemolithoautotrophic photoautotroph, photoheterotroph	Autotroph, chemoheterotroph
Source of isolation	Paper mill	Freshwater and terrestrial environments	Soil, sewage
Substrate utilization			
Citrate	–	V	–
Propionate	–	ND	+
L-Rhamnose	+	ND	–
L-Histidine	–	ND	+
L-Leucine	–	ND	+
Acid production from carbohydrates	–	+	+
Predominant fatty acid	C _{19:0} cyclo ω8c	C _{18:1} ω7c	C _{18:1} ω7c
DNA G+C content (mol%)	69.4–70.2	64–70	64–70

Ecology of *Rubellimicrobium*

The natural habitat of rubellimicrobia is so far unknown. Baumgartner *et al.* (2003) reported on a novel extremely thermophilic amoeba (*Echinamoeba thermarum*) that could be cultivated monoaxenically on a thermophilic, pink-pigmented alphaproteobacterium (isolate OSrt). The 16S rRNA gene sequence of the bacterium showed >99% similarity to those of our 'group IV' isolates. OSrt has been enriched as a food bacterium from a hot spring (Octopus Spring) in Yellowstone National Park, USA. The bacterium is strictly aerobic and grows between 25 and 55 °C. Based upon 16S rRNA gene sequence data, the hot-spring isolate OSrt can be certainly classified as a *Rubellimicrobium* sp. (Fig. 1). Comparative taxonomic investigations will show whether *Rubellimicrobium* sp. OSrt is a further strain of *Rubellimicrobium thermophilum* or represents a separate species of *Rubellimicrobium*.

Description of *Rubellimicrobium* gen. nov.

Rubellimicrobium (Ru.bel.li.mi.cro'bi.um. L. adj. *rubellus* -a -um somewhat red, reddish; N.L. neut. n. *microbium* microbe; N.L. neut. n. *Rubellimicrobium* reddish-coloured microbe).

Rod-shaped cells that stain Gram-negative; cells are sensitive to vancomycin, lyse in the presence of 3% (w/v) KOH and are L-alanine aminopeptidase-positive. Endospores are not formed. Red intracellular pigments (carotenoids) are produced. Strictly aerobic, cytochrome *c* oxidase-positive; catalase is weakly positive. Chemo-organotrophic; a large number of organic compounds are used for growth. Ubiquinone Q-10 is the major respiratory lipoquinone. Main phospholipids are diphosphatidylglycerol and phosphatidylcholine. Phosphatidylglycerol is present in minor amounts. Predominant fatty acids are *cis*-8-cyclo-nonadecanoic acid (C_{19:0} cyclo ω 8; the major component), C_{18:0} and C_{16:0}. The main components in the polyamine pattern are spermidine, *sym*-homospermidine and putrescine. The G+C content of the DNA is 69.4–70.2 mol% (by HPLC). Phylogenetically, a member of the order *Rhodobacterales* of the class *Alphaproteobacteria*. The type species is *Rubellimicrobium thermophilum*.

Description of *Rubellimicrobium thermophilum* sp. nov.

Rubellimicrobium thermophilum (ther.mo'phil.um. Gr. n. *therme* heat; Gr. adj. *philos* friendly to, loving; N.L. neut. adj. *thermophilum* heat-loving).

Cells are 0.6–0.8 × 2.0–4.0 µm. Motile by means of polar flagellation; number of flagella varies from one to three. Moderately thermophilic; temperature optimum is between 45 and 52 °C ($T_{\max}$ 56 °C). Slow growth at 28 and 37 °C. No growth at room temperature or at 57 °C. Colonies on R2A agar after 2 days incubation at 45 °C are translucent, circular, entire, convex, smooth and red-pigmented. The pigment absorbance spectrum (acetone/methanol extract)

is characterized by a major peak at 495 nm and two shoulders at 465 and 525 nm. Growth does not occur under anaerobic conditions in the dark or in the light. Bacteriochlorophyll *a* is not synthesized. Urease-positive. Nitrate is not reduced. The following compounds are assimilated: L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, α -D-melibiose, D-rhamnose, D-ribose, sucrose, salicin, D-trehalose, D-xylose, adonitol, *myo*-inositol, maltitol, D-mannitol, D-sorbitol, acetate, 4-aminobutyrate, glutarate, DL-3-hydroxybutyrate, DL-lactate, L-malate, oxoglutarate, pyruvate, L-alanine, L-ornithine and L-proline. Acid is not produced from D-glucose, lactose, sucrose, L-arabinose, L-rhamnose, maltose, D-xylose, cellobiose, D-mannitol, dulcitol, salicin, adonitol, *myo*-inositol, sorbitol, raffinose, trehalose, methyl α -D-glucoside, erythritol, melibiose, D-arabitol or D-mannose. The following compounds are not assimilated: N-acetyl-D-glucosamine, putrescine, propionate, *cis*- and *trans*-aconitate, adipate, azelate, citrate, fumarate, itaconate, mesaconate, suberate, β -alanine, L-aspartate, L-histidine, L-leucine, L-phenylalanine, L-serine, L-tryptophan, 3- and 4-hydroxybenzoate and phenylacetate. The following compounds are hydrolysed: *p*-nitrophenyl (pNP) α -D-glucopyranoside, pNP β -D-glucopyranoside, bis-pNP phosphate, pNP phenylphosphonate and L-alanine *p*-nitroanilide (pNA). Aesculin, pNP β -D-galactopyranoside, pNP β -D-glucuronide, pNP phosphorylcholine, 2-deoxythymidine-5'-pNP phosphate, L-glutamate- γ -3-carboxy pNA, L-proline pNA, Tween 80, starch and casein are not hydrolysed. Susceptible to ampicillin, chloramphenicol, colistin sulphate, gentamicin, kanamycin, lincomycin, neomycin, nitrofurantoin, penicillin G, polymyxin B, streptomycin, tetracycline and vancomycin. Chemotaxonomic characteristics are the same as those given in the genus description. Polyamine patterns and fatty acid compositions of individual strains are summarized in Supplementary Tables S1 and S2. The DNA G+C content of the type strain is 70 mol% (by HPLC).

The type strain is strain C-lvk-R2A-2^T (=CCUG 51817^T = DSM 16684^T = HAMBI 2421^T), isolated from coloured deposits in a pulp dryer in Finland.

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