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-nitrilopropionamide, 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-2T, 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 33485T, 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 C19:0cyclo (39–44%), C18:0 (21–24%) and C16:0 (21–23%). Fatty acids present in smaller amounts included C18:1ω7c, C10:0 3-OH, C18:1ω7c 11-methyl, C20:0 9c and C17:0 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-2T (=CCUG 51817T =DSM 16684T =HAMBI 2421T) 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 runnability 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.,...
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 geothermatis (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-2T and C-R2A-5d were almost indistinguishable; isolate C-R2A-5d showed minor differences. According to UPGMA clustering with the Dice coefficient, the riboprints of isolates C-lvk-R2A-1, C-lvk-R2A-2T, 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-2T, 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 fasta3 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 Alphaproteobacteria (Garrity et al., 2005, 2006). The highest 16S rRNA gene sequence similarities were found to Rhodobacter blasticus ATCC 33485T (94-7–94-9 %) and Paracoccus kocurii JCM 7684T (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 33485T. Bootstrap analysis gave 100 % confidence for this branching.

The DNA base compositions, analysed by HPLC (Cashin et al., 1977; Mesbah et al., 1989; Tamaoka & Komagata, 1984), of isolates C-R2A-lvk-2T, 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<sub>19:0</sub> cyclo ω8c (39–44 %), C<sub>18:0</sub> ω7c (21–22 %), C<sub>17:0</sub> (20–28 %) and C<sub>18:1</sub> ω9c (20–29 %). 

---

[16S rRNA gene-based phylogeny, riboprint patterns and base composition of DNA](#)
This fatty acid signature differed from those of the majority of species in the order Rhodobacterales mainly in the proportions of C19 : 0 cyclo\textsubscript{8}c and C18 : 1\textsubscript{7}c. Interestingly, the ‘group IV’ isolates produced cis-8-cyclo-nonadecanoic acid (C19 : 0 cyclo\textsubscript{8}c) as the major (>35 %) fatty acid. Consulting published data, significant amounts of cyclopropane (C19 : 0) acids are typical of species of the order Rhizobiales such as Rhodovarius lipocyclicus (Kämpfer et al., 2004), Martellella mediterranea (Rivas et al., 2005), Ochrobactrum gallinicae (Kämpfer et al., 2003) and Pleomorphomonas oryzae (Xie & Yokota, 2005), some rhizobia (Tighe et al., 2000) and of facultative serine-pathway methyllobacteria (Doronina et al., 2000). Within the order Rhodobacterales, C19 : 0 cyclo\textsubscript{8}c as a major component has been reported for Jannaschia helgolandensis and Oceanicola batsensis (Wagner-Doellner 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, phosphatidyl-
choline and an unknown aminolipid. Additionally, phos-
phatidylglycerol was detected in minor amounts. The
majority of these lipids have been detected in a multitude
of taxa within the Alphaproteobacteria. Phosphatidyletha-
nolamine, commonly present in Gram-negative bacteria,
was not detected in lipid extracts of the ‘group IV’ isolates.
Within the Rhodobacteraceae, the absence of phosphatidyl-
ethanolamine has also been reported for Antarcobacter
heliothermus, Roseobacter denitrificans, Roseobacter litoralis,
Roseisalantaricus 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
\( T_{\text{max}} \) was determined by incubating inoculated R2A
agar plates in a Stuart scientific biological incubator
S101D (Bibby Sterilin) with gradual temperature incre-
ments 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 tem-
perature 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 \( x1000 \)).
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
Plioform 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 trans-
lucent, 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 \((\sim0.6–0.8 \times
2.0–4.0 \mu m)\), 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-prosthecate,
curved cell-surface structures (Fig. 2). Cells were partially
attached to self-secreted polymeric substances (Supple-
mental Figs S2 and S3). Intracellular inclusion bodies of
polyphosphate and polyhydroxyalkanoates were found in
the cytoplasm of the majority of cells. Whether the curved
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 tryp-
tone 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; \( T_{\text{max}} \) 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
Rha. 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', 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

E. B. M. Denner and others
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-2T, 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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>Rubellimicrobium</em></th>
<th><em>Rhodobacter</em></th>
<th><em>Paracoccus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigment production</td>
<td>+</td>
<td>+</td>
<td>v</td>
</tr>
<tr>
<td>Cell shape</td>
<td>Rods</td>
<td>Ovoid to rod-shaped</td>
<td>Coccoid to short rods</td>
</tr>
<tr>
<td>Intracytoplasmic membranes</td>
<td>–</td>
<td>+</td>
<td>v</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>v</td>
<td>–</td>
</tr>
<tr>
<td>Optimum growth temperature (°C)</td>
<td>45–54</td>
<td>30–35</td>
<td>25–42</td>
</tr>
<tr>
<td>Mode of respiration</td>
<td>Aerobic</td>
<td>Faculative</td>
<td>Aerobic</td>
</tr>
<tr>
<td>Bacteriochlorophyll a</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth physiology</td>
<td>Chemoheterotroph</td>
<td>Chemolithoautotrophic, photoautotrophic, photoheterotroph</td>
<td>Autotroph, chemoheterotroph</td>
</tr>
<tr>
<td>Source of isolation</td>
<td>Paper mill</td>
<td>Freshwater and terrestrial environments</td>
<td>Soil, sewage</td>
</tr>
<tr>
<td>Substrate utilization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>–</td>
<td>v</td>
<td>–</td>
</tr>
<tr>
<td>Propionate</td>
<td>–</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>–</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>–</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from carbohydrates</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Predominant fatty acid</td>
<td>C_{19:0} cyclo o8c</td>
<td>C_{18:1} o7c</td>
<td>C_{18:1} o7c</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>69.4–70.2</td>
<td>64–70</td>
<td>64–70</td>
</tr>
</tbody>
</table>

**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.
 Ecology of Rubellimicrobium

The 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.bell.i.miкроbi.ум). 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. Chemorganotrophic; a large number of organic compounds are used for growth. Ubiquinone Q-10 is the major respiratory lipoquinone. Main phospholipids are diphosphatidylglycerol and phosphatidylethanolamine. Phosphatidylethanolamine is present in minor amounts. Predominant fatty acids are cis-8-cyclo nonadecanoic acid (C19:1 cyclo ω8c; the major component), C18:0 and C16:0. 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.mо φil.ум. Gr. n. therme heat; Gr. adj. philos friendly to, loving; N.L. neut. adj. thermophilum heat-loving).

Cells are 0.6–0.8 x 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 (Tmax 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, D- and L-melibiose, D-rhamnose, D-ribose, sucrose, salicin, D-trehalose, D-xyllose, adonitol, myo-inositol, maltitol, D-mannitol, D-sorbitol, acetate, 4-amino butyrate, 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-xyllose, cellobiose, D-mannitol, dulcitol, salicin, adonitol, myo-inositol, sorbitol, raffinose, trehalose, methyl 2-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 ρ-D-galactopyranoside, pNP β-D-glucuro nide, 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-2T (=CCUG 51817T = DSM 16684T = HAMBI 2421T), isolated from coloured deposits in a pulp dryer in Finland.

Acknowledgements

The Nationwide Graduate School for Applied Biosciences (ABS), Kemira Oyj, National Technology Agency (TEKES) and the Finnish paper industry supported this study. TEKES grant to Shines-Pro projekt. We thank Riitta Saastamoisen (HAMBI) for technical assistance and are grateful to Professor H. G. Trüper, University of Bonn, for etymological evaluation of the taxon name. Analyses of base composition of DNA and 16S rRNA gene sequencing were performed by the Identification Service of the DSMZ.

References


http://ijs.sgmjournals.org


