**NOTE**

_Rhodanobacter lindaniclasticus gen. nov., sp. nov., a lindane-degrading bacterium_

Renaud Nalin,1 Pascal Simonet,1 Timothy M. Vogel2 and Philippe Normand1

Author for correspondence: Renaud Nalin. Tel: +33 4 72 44 80 00. Fax: +33 4 72 43 12 23.
e-mail: nalin@biomserv.univ-lyon1.fr

Lindane-degrading activity under aerobic conditions has been observed in two bacterial strains: UT26, phenotypically identified as _Sphingomonas paucimobilis_, and a new single unidentified isolate named RP5557T. The _rrs_ (16S rDNA) sequences for both strains and the phenotypic characteristics for the unidentified isolate RP5557T were determined. RP5557T does not have high identity (less than 90% in all cases) with any sequence in the GenBank or RDP databases. A phylogenetic analysis based on _rs_ sequences indicated that RP5557T belongs to the _γ-Proteobacteria_ in a coherent phylum that includes the genera _Xanthomonas_ and _Xylella_ (100% bootstrap), whereas UT26 is clearly separate from the _Xanthomonas_ cluster. Based on the phylogenetic analyses and on the phenotypic characteristics, a new genus, _Rhodanobacter_, containing a single species, _Rhodanobacter lindaniclasticus_, is proposed for strain RP5557T (=LMG 18385T), which becomes the type strain.

**Keywords:** phylogeny of lindane degraders, soil, gamma-hexachlorocyclohexane

Gamma-hexachlorocyclohexane (γ-HCH or lindane) has been used for many years as a plant protection product in agriculture and for wood treatment. Highly chlorinated aliphatic compounds such as lindane are often degraded relatively slowly in many subsurface environments, such as soil, sediment and groundwater (Vogel et al., 1987). HCH isomers have been found to persist in some environments for more than 15 years (Voerman & Besemer, 1970). Nevertheless, lindane biodegradation has been observed in both anaerobic and aerobic ecosystems (Bhuyan et al., 1992; Jagnow et al., 1977; Wada & Peel, 1989).

Few bacterial isolates have been described as able to mineralize this compound. One is an anaerobic _Clostridium_ sp. (MacRae et al., 1969; Ohisa et al., 1980) and two are aerobic bacteria. One of the latter phenotypically resembles a _Sphingomonas paucimobilis_ strain (UT26) isolated from a Japanese soil (Senoo & Wada, 1989) and the other was isolated from a French soil from a wood treatment site and named RP5557T. Other attempts to isolate lindane-degrading bacteria from different soils have been unsuccessful. Despite the geographical distance between the isolation sites of the two bacteria, the activity of γ-HCH dehydrochlorinase, the enzyme that catalyses the first step in the lindane degradation pathway, is encoded by a gene, _linA_, that is highly conserved (99.4% identity) between these two strains (Thomas et al., 1996).

The aim of this study was to determine the taxonomic position of the French aerobic lindane-degrading bacterium (RP5557T) using phenotypical characteristics and _rrs_ (16S rDNA) sequence analysis. The results provide evidence that RP5557T is representative of a new bacterial genus.

**Morphological and physiological characteristics**

The RP5557T strain was isolated on Standard Methods Agar (SMA) plates containing, in g l⁻¹: Bio trypcase (bioMérieux), 5; yeast extract, 2.5; and glucose, 1; pH 7.0. The strain was isolated from a soil sample from a wood treatment site and was enriched by keeping it for over 18 months in a flask containing only lindane as a carbon source (Thomas et al., 1996). More experiments from the same and other soils were carried out in an attempt to isolate other lindane-degrading bacteria, but these were unsuccessful. This enrichment procedure suggests that RP5557T is not present in large quantities in soil even after long-term contamination or that it is not competitive among the culturable soil bacterial community.

**Abbreviation:** γ-HCH, gamma-hexachlorocyclohexane.

The complete primary sequences of the _rrs_ (16S rDNA) genes of _Rhodanobacter lindaniclasticus_ and _Sphingomonas_ sp. UT26 have GenBank accession numbers AF039167 and AF039168, respectively.
Table 1. Phenotypic and biochemical characteristics of Rhodanobacter lindaniclasticus

<table>
<thead>
<tr>
<th>Character</th>
<th>RP5557T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>Negative</td>
</tr>
<tr>
<td>Spore production</td>
<td>Negative</td>
</tr>
<tr>
<td>Capsule formation</td>
<td>Negative</td>
</tr>
<tr>
<td>Branching</td>
<td>Negative</td>
</tr>
<tr>
<td>Pigment on King A and B media</td>
<td>Negative</td>
</tr>
<tr>
<td>Growth at:</td>
<td></td>
</tr>
<tr>
<td>4 °C</td>
<td>Negative</td>
</tr>
<tr>
<td>30 °C</td>
<td>Positive</td>
</tr>
<tr>
<td>41 °C</td>
<td>Positive</td>
</tr>
<tr>
<td>Motility</td>
<td>Negative</td>
</tr>
<tr>
<td>Lipid inclusion</td>
<td>Negative</td>
</tr>
<tr>
<td>Respiration</td>
<td>Strictly aerobic</td>
</tr>
<tr>
<td>Indole formation</td>
<td>Negative</td>
</tr>
<tr>
<td>Glucose fermentation</td>
<td>Negative</td>
</tr>
<tr>
<td>G+C content (mol%)</td>
<td>63</td>
</tr>
</tbody>
</table>

Strain RP5557T was found to form dark-yellow, flat colonies with clean edges on SMA medium agar plates. The yellow pigment exhibited the characteristic absorbance spectrum of the carotenoid nothoxanthin (Thomas et al., 1996). Strain RP5557T was found to form non-sporulated, non-capsulated straight Gram-negative rods. Phenotypic characteristics were determined using Pasteur Pseudomonas strips (Institut Pasteur), the API 20 NE microtube system and API 50 CH strips (API bioMérieux) as recommended by the manufacturers. The results are indicated in Table 1. The bacterium grew in 24 h at 30 °C and 41 °C but not at 4 °C. The mean G+C content of RP5557T was determined by HPLC (Peyret et al., 1989) and was found to be 63 mol %. This strain is strictly aerobic and does not reduce nitrite or nitrate. It exhibits catalase and oxidase activities and can use lindane as the sole carbon source (Thomas et al., 1996).

Comparison of the morphological and biochemical characteristics of RP5557T with those of previously described bacteria present in the phenotypic databases suggested that RP5557T should be grouped with the genus Pseudomonas as defined in the API and Pasteur phenotypic databases. However, the RP5557T phenotype has not yet been described in these phenotypic databases. Nevertheless, the phenotypic characteristics suggested that RP5557T belongs to the γ-Proteobacteria. In any case, a more precise identification at the genus and species levels requires a phylogenetic approach such as that based on 16S rDNA sequence analysis.

Amplification of rrs (16S rDNA) sequences and cloning

Chromosomal DNAs from the two aerobic lindane-degrading isolates, strains UT26 and RP5557T, were obtained from pure cultures (Brenner et al., 1982). Approximately 0.3 μg of total DNA was used for PCR amplification. The primers used to amplify the whole rrs gene, permitting subsequent cloning in a directional manner, included primer FGPS-255 (5'-TGGGAAAGCTTGTACCTGCTGCT-3'), which contains a HindIII restriction site, and primer FGPS1509-153 (5'-AAGGAGGGGATCAGCCTCCAGC-3'), which contains a BamHI restriction site (the restriction sites are in bold) (Normand et al., 1996). PCRs were carried out in 0.2 ml Eppendorf tubes in a final volume of 100 μl reaction mixture containing template DNA, reaction buffer [10 mM Tris/HCl (pH 8.3), 1.5 mM MgCl2, 50 μM KCl, 10 % (w/v) gelatin], each dNTP at a concentration of 200 μM, 0.5 μM oligonucleotides and 2 U TaqI DNA polymerase (Gibco BRL). DNA amplification was carried out in a thermocycler (Perkin-Elmer) using the following programme: initial denaturation for 3 min at 94 °C, 35 cycles of denaturation (30 s at 95 °C), annealing (30 s at 55 °C) and extension (1 min at 72 °C) and final extension (3 min at 72 °C). To analyse the amplification products, 5 μl of each reaction mixture was separated on a 1% agarose gel (Nusieve; FMC) and visualized using 1 mg ethidium bromide 1-1 solution. Amplified fragments were a single product of the expected size, approximately 1500 bp. Purification of the amplified products was achieved by extracting the band from the gel and purified on the Quiaex II kit (Qiagen). The amplified fragments were digested with BamHI and HindIII, cloned into BamHI/HindIII-cut pBluescript II SK vector (Stratagene) and transformed into Escherichia coli DH5αF' (Gibco BRL).

Sequencing and phylogenetic analysis

At least five clones were pooled to minimize errors due to either TaqI polymerase amplification or differences between copies of the rrs (16S rDNA) gene. The sequences were obtained for both strands by using primers FGPS485-292 CACGAGCGCCGATTAAA, FGPS747-293 AAGCAGGATTAGATAC, FGPS1047-295 ATGTGGTGTTAAGTGC, FGPS910-270 AGCCTGGCGCGCTCCTCC, FGPS1176-112 GG- GCAGATGACCTTGCACGTG, FGPS505-313 GT-
Rhodanobacter lindaniclasticus gen. nov., sp. nov.

Fig. 1. Rooted phylogenetic tree based on the rrs (16S rDNA) sequences of Rhodanobacter lindaniclasticus strain RP5557\(^\text{T}\) and related bacteria and Sphingomonas sp. UT26. This tree was made using the neighbour-joining method (Saitou & Nei, 1987) with a Kimura (1983) two-parameter distance matrix and the no-gap option. Numbers indicate the frequency of occurrence (%) in 1000 bootstrapped trees. P and L indicate those results confirmed by the parsimony (Fitch, 1971) and maximum-likelihood (Olsen et al., 1994) methods, respectively. The bar represents 23 nucleotide substitutions per 100 nucleotides.

ATTACCGCGGCTGCTG, as well as primers T3 and T7 (Pharmacia), which were necessary to determine the complete rrs sequences (Normand et al., 1996).

Comparison of the UT26 rrs sequence with the GenBank database using the BLASTN program (Altschul et al., 1990) and RDP (Ribosomal Data Project) (Maidak et al., 1997) revealed that the most closely related bacterial species belong to the Sphingomonas genus. The closest species was found to be Sphingomonas chlorophenolica ATCC 33790 (X87161) with 98\% identity between positions 25 and 1416.

Using the same procedure, identity between RP5557\(^\text{T}\) and the closest sequence, the Xanthomonas campestris rrs sequence (results not shown), was found to be only 90\%. It has been suggested that in bacterial strains with less than 97\% rrs sequence identity the DNA-DNA hybridization level is less than 70\% (Stackebrandt & Goebel, 1994), which defines genomic species (Wayne et al., 1987). Thus, based on the rrs sequence analyses, new taxons could be detected. In the absence of any close homologous sequence in the GenBank database and the RDP (90\% or less sequence identity), phylogenetic relationships were investigated using representatives of \(\gamma\)-Proteobacteria genera and rooted using the Sphingomonas rrs sequences. Thirty-two representative \(\gamma\)-Proteobacteria sequences were chosen for the phylogenetic analysis.

The rrs sequences were aligned and compared using the multiple-alignment CLUSTAL W algorithm (Thompson et al., 1994). The evolutionary distance sequences were computed based on the no-gap option and using the Kimura two-parameter model (which assumes that transitions and transversions occur at different rates) (Kimura, 1983) with the PHYLO_WIN package (Galtier et al., 1996). To estimate the phylogenetic relationships among the organisms, a tree (Fig. 1) was derived using
the neighbour-joining method (Saitou & Nei, 1987). The topology of the tree was tested by performing 1000
bootstrap resamplings of the data (Felsenstein, 1985) and comparing the results of the parsimony (Fitch,
1971) and maximum-likelihood (Olsen et al., 1994) methods. The analysis confirmed that UT26 is clus-
tered into a coherent group with the Sphingomonas spp. (bootstrap 100%). Despite the phenotypic char-
acteristics, which permitted positioning UT26 as a Sphingomonas paucimobilis, the closest phylogenetic
neighbour was found to be Sphingomonas chloro-
phenolica.

Strain RP5557T was found to be distinct from all other
known bacteria in the γ-Proteobacteria group. The
position of RP5557T at the root of the branch, con-
taining Xanthomonas, Xylella and Stenotropho-
monas genera, was validated by 100% bootstrap and by the maximum-likelihood (Olsen et al., 1994) and parsimony analyses (Fitch, 1971) (Fig. 1). Aning
according to the sequence phylogenetic analyses, the closest genera are Xanthomonas and Stenotrophomonas, but they are distant enough (90-92% similarity) to war-
rant placing RP5557T into a new genus containing a
single species, which is also supported by the pheno-
typic characteristics (Table 1). We propose that,
because its particular specific degradative activity
and its isolation as a novel organism within this system,
the bacterium should be called Rhod-
abacter lindaniclasticus.

Description of Rhodanobacter gen. nov.

Rhodanobacter (Rho.da`no.bac.ter. L. masc. n. Rhod-
anus River Rhône; M.L. masc. n. bacter equivalent of
bacterium, a small rod; M.L. masc. n. Rhodanobacter
rod isolated close to the River Rhône).

Yellow colonies of diameter 0.2–1 mm with clean edges
on SMA agar plates after 3 d at 30 °C. Non-motile,
Gram-negative rods. No spores are formed and no
ramification occurs. Aerobic, chemo-organotrophic
and catalase- and oxidase-positive. Maximum growth
at 30 °C.

Description of R. lindaniclasticus sp. nov.

Rhodanobacter lindaniclasticus (lin.da`ni.clas.ti.cus. Fr.
masc. n. lindane commercial name of γ-HCH; Gr.
adj. elasticus breaking, from Gr. part. perf. klastos
broken; M.L. masc. adj. lindaniclasticus lindane-
breaking).

Optimum temperature for growth 30 °C. Colonies are
yellow (nothoxanthin), circular, 0.2–1 mm diameter
and convex on nutrient agar plates. Grows on Millie
Base Salin (MBS) medium with γ-HCH as sole carbon
source. Tests for catalase, oxidase and Tween 80
esterase are positive. Urease, protease, tryptophanase,
nitrate reductase, amylase and DNase are negative.
Carbon sources utilized are D-glucose, ascorcin, cel-
biose, trehalose, D-xylose, lactate, caprate, malate and
citrate. G+C content of the DNA is 63 mol%. The
type strain is RP5557T (Table 1) (=LMG 18385T).

Acknowledgements

We thank J. Haurat (UCB, Lyon, France) for technical
assistance in the sequencing work, H. Meugnier (Hospital E.
Herriot, Lyon) for G+C content determination and Dr Y.
Nagata (University of Tokyo, Japan), who provided the
UT26 strain for comparison.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J.
403–410.

Accelerated aerobic degradation of γ-hexachlorocyclohexane in
suspensions of flooded and non flooded soils pretreated with

Brenner, D. J., McWorter, A. C., Knuston, J. K. L. & Steigerwalt,
teriacae associated with human wounds. J Clin Microbiol 15,
1133–1140.

approach using the bootstrap. Evolution 39, 783–791.

Fitch, W. M. (1971). Toward defining the course of evolution: a
minimum change for the specific tree topology. Syst Zool 20,
406–416.

WIN: two graphic tools for sequence alignment and molecular
phylogeny. CABIOS 12, 543–548.

dehlorination and degradation of hexachlorocyclohexane iso-
mers by anaerobic and facultative anaerobic bacteria. Arch

Cambridge: Cambridge University Press.

degradation of the insecticide Lindane by Clostridium sp. Nature
221, 859–860.

Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey,

Normand, P., Orso, S., Cournoyer, B., Jeannin, P., Chapelon, C.,
phylogeny of the genus Frankia and related genera and
emendation of the family Frankiaceae. Int J Syst Bacteriol 46,
1–9.

Ohisa, N., Yamaguchi, M. & Kuriara, N. (1980). Lindane degra-
dation by cell-free extracts of Clostridium rectum. Arch Micro-
biol 125, 221–225.

fastDNAml: a tool for construction of phylogenetic trees of
DNA sequences using maximum likelihood. Comput Appl
Biosci 10, 41–48.

Determination of G+C content of DNA using HPLC for
identification of staphylococci and micrococci. Res Microbiol
140, 467–475.


International Journal of Systematic Bacteriology 49


