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Title:

***Rheinheimera perlucida* sp. nov., a novel marine bacterium of the *Gammaproteobacteria* isolated from surface water of the central Baltic Sea**

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SUMMARY

A bacterial isolate from the Baltic Sea, BA131^T, was characterized for its physiological and biochemical features, fatty acid profiles, its G+C content and its phylogenetic position based on 16S rRNA gene sequence comparison. The strain was isolated from the surface water of the central Baltic Sea during the decay of a plankton bloom. Phylogenetic analyses of the 16S rRNA gene sequence revealed a clear affiliation with the *Gammaprotebacteria*, and showed closest phylogenetic relationship with the genera *Alishewanella* and *Rheinheimera.* The G+C-content of the DNA was 48.9 mol%. The strain was non-pigmented, Gram-negative, rod-shaped, motile by means of a single polar flagellum, catalase and oxidase positive. Growth was observed at salinities from 0 to 8%, with an optimum at 1-3%. Temperature for growth ranged from 4 to 37 °C, with an optimum around 25 °C. The fatty acids were dominated by unsaturated fatty acids (>61%); high abundance fatty acids were of 16:0 (17-18%), 16:1 omega 7c (24-33%), 17:1 omega 8c (14-18%), and 18:1 omega 7c (9-12%). According to its fatty acid composition, and the phylogenetic analysis based on the 16S rRNA gene sequence, the described strain BA131^T is suggested as representative of a new species of the genus *Rheinheimera* and the name *Rheinheimera perlucida* sp. nov. is suggested for the Baltic Sea isolate, for which the type strain is BA131^T (DSM XXX, LMG XXX, CIP XXX).

The isolate was obtained from surface water of the central Baltic during the decay of a late summer phytoplankton bloom (Brettar *et al.,* 2006). *Gammaproteobacteria* are considered to represent a large fraction of the marine surface water bacteria able to grow and degrade rapidly the more easily degradable organic fraction of the marine organic matter (Bianchi & Bianchi, 1995; Pinhassi & Berman, 2003; Poretsky *et al.* 2005). Recent studies also show this role and a higher abundance for the genus *Rheinheimera* in marine and estuarine environments (Alavi *et al.,* 2001, Giovannoni & Stingl, 2005). The new isolate complements a very recent branch of the *Gammaproteobacteria*, that is so far composed out of the genera *Alishewanella* (Fonnesbech-Vogel *et al*. 2000), *Rheinheimera* (Brettar *et al*. 2002, Romanenko *et al*., 2003), and *'Alkalimonas'* (Ma *et al*. 2004). Most of the isolates and environmental 16S rRNA sequences of this branch are of marine or aquatic origin, with the exception of *Alishewanella fetalis* and a few environmental sequences in its closer phylogenetic neighbourhood.

Strain BA131^T was isolated during a cruise onboard RV "Aranda" in September 1998 from surface water (5m, 15 °C, 7‰ S, pH 8.4) from a site in the Central Baltic Sea, i.e., station TEILI1 (59.2607° N, 21.3002° E). All details on environmental conditions, sampling and isolation procedures are given elsewhere (Brettar *et al.,* 2002, Brettar & Rheinheimer, 1992; Brettar & Höfle, 1993; Höfle & Brettar, 1995). Medium for isolation was ZoBell agar (Oppenheimer & Zobell, 1952). The strain grew well on half strength ZoBell agar and marine broth or agar (Difco, No.2216).

The isolate was tested for a number of key characteristics using standard procedures (Gerhardt *et al.,* 1994), such as KOH string test (Gram behaviour), cell size and morphology (phase contrast microscopy, electron microscopy after Pt/C shadow casting), cytochrome oxidase, and catalase (3% H_2O_2). Furthermore, production of hydrogen sulphide (Dye 1968), aminopeptidase (Merck Bactident test), haemolysis, hydrolysis of starch, gelatin, Tween 80, and lecithine were tested.

The strain was additionally characterised by the whole test spectrum of the identification systems API 50CH, API 20NE, API ZYM (bioMérieux), and BIOLOG GN2 at 28 °C. Growth at different temperatures was assessed at 4, 10, 20, 25, 30, 33, 37 and 40°C. Growth at different salinities was tested at 0, 0.8, 1.0, 1.5, 3, 6, 8 and 10% salinity. Growth at different pH was tested for pH 5.7, 7, 9 and 10 (pH adjusted using bicarbonate buffer or HCl, growth assessed by occurrence of visible colonies on agar). For these tests we used half strength marine broth or agar (Difco 2216), except for the salinity test where half strength salt-free ZoBell medium was supplemented with the respective amount of NaCl.

Genomic DNA was prepared from individual colonies as described by Moore *et al*. (1996). 16S rRNA genes were amplified by PCR (Mullis & Faloona, 1987) and the PCR-products were sequenced directly as described previously (Moore *et al*., 1999).

For phylogenetic analysis based on the 16S rRNA gene sequence, most similar sequences were gathered by running BLAST queries on servers of NCBI (nr database), EBI (EMBL database) and Infobiogen (NucAll database) with filter option set to false; these queries returned a total of 98 most similar sequences having a 16S rRNA sequence well described in the Feature lines. These sequences were included and aligned into a local database of 122 000 already aligned and analyzed bacterial 16S rRNA gene sequences. In a first analysis, these 98 sequences were carefully aligned (manual adjustments) and analyzed by phylogeny (BIONJ as explained below). This allowed to select a set of 28 sequences belonging to a clade including the BA131^{T} sequence. These 28 sequences were further analyzed using three phylogenetic methods (bioNJ, Maximum Likelihood and Maximum Parsimony). For the NJ analysis, distance matrices were calculated using the Kimura two parameters correction, bioNJ was used according to Gascuel (1997), maximum likelihood and maximum parsimony were from PHYLIP (Phylogeny Inference Package, version 3.573c, distributed by J.

Felsenstein, Department of Genetics, UW, Seattle, WA, USA). The phylogenetic trees were drawn using NJPLOT (Perrière & Gouy 1996). Bootstrap analyses was performed using BIONJ and 1000 replications. From the 28 sequences, 8 sequences obtained from cultured strains (including the 6 sequences of the type strains of this branch) plus the environmental clone sequence most related to the BA131^T sequence were chosen for a final analysis; all three methods and a bootstrap analysis were done as described above, using positions 159-1445 of the BA131^T sequences (no problem of alignment, and no missing part in any sequence). The result of this final phylogenetic analysis is shown in Figure 2.

The DNA G+C content (mol%) of the strains was determined using HPLC analysis of hydrolysed DNA according to Tamaoka & Komagata (1984) and Mesbah *et al*. (1989).

For analysis of the cellular fatty acid profile, the strain was grown on half strength marine agar (Difco No.2216) 24 hrs at 28 °C. For comparison with *Alishewanella fetalis*, BA131^T and the type strain of *Rheinheimera baltica* OSBAC1^T were grown additionally at 30°C for 2 days on blood agar. The fatty acid methyl esters (FAMEs) were obtained from washed cells by saponification, methylation and extraction. Analysis by gas chromatography was controlled by MIS software (Microbial ID Newark DE, U.S.A.) and peaks were automatically integrated and identified by the Microbial Identification software package (Sasser, 1990).

Strain BA131^T was Gram-negative, formed rods (Fig. 1a,b IJSEMonline), and non-pigmented transparent colonies. All details on morphological. physiological and biochemical traits are summarized in the species description plus Table 1a (IJSEM online). In general, BA131T showed a rather limited potential of substrate use in the API 50CH, API 20NE and Biolog GN2 test systems. By contrast, the APIZYM test, revealed a rather broad set of 9 enzymatic

activities. As a general rule, phenotypic features were rated positive when a signal was obtained either weak or more pronounced (for details see Table 1a, IJSEM online).

In terms of phenotypic features, BA131^T could be differentiated from *Rheinheimera baltica* by the the pigment of the colonies, reduction of nitrate, the salt tolerance, acid production from various carbohydrates (D-glucose, cellobiose, maltose, gentobiose), assimilation of glucose and maltose, four enzymatic activities, and utilization of seven substrates (Table 1). From *R. pacifica*, BA131^T could be differentiated by the number and insertion site of the flagella, the reduction of nitrate, activity of acidic phosphatase, assimilation of arabinose, citrate and maltose, the utilization of three amino acids, plus acetic acid and glycerol. BA131^T could be differentiated from the genus *Rheinheimera* by the reduction of nitrate, acidic phosphatase and the assimilation of maltose. BA131^T could be differentiated from *Alishewanella fetalis* by the presence of a flagellum, the need for NaCl for growth, the temperature optimum, the temperature range for growth, the reduction of nitrite, thiosulfate and TMAO, hydrolysis of starch and assimilation of N-acetylaglucosamine. Compared to the genus *'Alkalimonas'*, BA131^T had a different temperature range and optimum for growth, a different pH range and optimum for growth, and differed by the use of 12 substrates (Table 1). In general, BA131^T had a rather restricted spectrum of used organic substrates.

All phylogenetic analyses based on 16S rRNA gene sequences and notwithstanding which other sequences were included in these analyses, revealed that BA131^T formed a very robust clade with *Alishewanella fetalis* and the genus *Rheinheimera* (all methods, very high bootstrap percentages), with the genus *'Alkalimonas'* as closest outgroup (Fig. 1). However, within this clade, the position of the BA131^T was somehow unstable, being clustered with *Rheinheimera* or *Alishewanella*, depending upon the outgroups chosen and the method used to construct a phylogenetic tree. This might indicate that BA131^T may represent a new genus.

With *Serratia entomophila* as outgroup, BA131^T clustered more closely with the genus *Rheinheime*ra (Fig. 1). In terms of 16S rRNA gene sequence similarity, BA131^T showed the highest similarity of 98% with a bacterium observed in methane-hydrate bearing deep marine sediments (AY093457, uncultured bacterium clone MB-A2-102; Reed *et al*. 2002*)*. Compared with strains of the species *Rheinheimera baltica* (AJ441080, AJ441082, AJ002006), BA131^T had sequence similarities ranging between 96.6 and 95.5%, with *Rheinheimera pacifica*, a similarity of 96.4%, respectively.

The G+C content analysed for strain BA131^T was 48.9 mol% (Table 1). The G+C contents of the related species *R. baltica, R. pacifica* and *A. fetalis* ranged from 48 to 51% and are therefore within the same range as the here described strain.

Table 2 shows the fatty acid composition of BA131^T and the phylogenetically related species *Rheinheimera baltica, R. pacifica, Alishewanella fetalis, 'Alkalimonas amylolytica'* and *'A. delamerensis'*. To enable a comparison with *Alishewanella fetalis*, fatty acids are given for *Rheinheimera baltica* OSBAC1^T and BA131^T after growth on blood agar (30°C, 2 d) additionally to cultivation on marine agar (half strength marine agar, 28°C). Major fatty acid components (> 10%) of BA131^T were 16:0, 16:1 omega 7c, 17:1 omega 8c and 18:1 omega 7c. Unsaturated fatty acids formed the major fraction of the total fatty acids representing 63.7% on marine agar, and 61.4% on blood agar. In terms of comparability with related species, the fatty acid composition of BA131^T showed the highest similarity with *Rheinheimera baltica* (e.g. for the major components 16:0, 16:1 omega 7c). A major distinction of BA131^T is the lower abundance of 17:1 omega 8c for *R. baltica*. The overall pattern of the fatty acid composition of BA131^T for the two growth conditions (marine agar, blood agar) was comparable; the major change was the disappearance of 15:0 on blood agar that was also the case for *R. baltica* OSBAC1^T. Based on the fatty acid composition after

growth on blood agar, *A. fetalis* could be differentiated from BA131^T and *R. baltica* by the presence of 15:0, and lower concentrations of 16:0.

CONCLUSION

Based on 16S rRNA gene sequence analysis, the Baltic Sea strain BA131^T is phylogenetically most closely related to the genera *Rheinheimera* and *Alishewanella* (Fig. 1) with a certain instability for its attribution to either of these genera. The fatty acids show a comparable pattern for BA131^T and the genera *Rheinheimera* and *Alishewanella* on the one hand. On the other hand, distinction of BA131^T and *Rheinheimera baltica* from *A. fetalis* is possible based on the two fatty acids 15:0 and 16:0. Features such as the occurrence of a flagellum, the temperature and salt range and optimum for growth, the spectrum of utilizable electron acceptors and organic substrates differentiate BA131^T from *A. fetalis,* and these features are not consistent with the description of the genus *Alishewanella* Fonnesbech Vogel *et al*. 2000. On the other hand, BA131^T could be differentiated from the phenotypic traits of the genus *Rheinheimera* by only three traits, i.e. the reduction of nitrate, acidic phosphatase and the assimilation of maltose, and fits well into the genus description. By inclusion of BA131^T in the genus *Rheinheimera*, the genus *Alishewanel*la can be distinguished by the presence of flagella, the salinity range for growth, the need for NaCl, the temperature spectrum for growth (growth at 4°, 10°, and 41°C), the use of thiosulfate and nitrite as electron acceptors, the assimilation of N-acetyl-glucosamine, and the hydrolysis of starch. Based on the polyphasic analyses and their results for the novel Baltic Sea isolate, we propose to assign strain BA131^T to the genus *Rheinheimera*, as the type strain of the novel species, i.e. *Rheinheimera perlucida*.

Description of *Rheinheimera perlucida* sp. nov.

Rheinheimera perlucida (per.lu'ci.da. L. fem. adj. *perlucida* meaning transparent, referring to the transparent and colourless colonies, to distinguish *R. perlucida* from the type species *Rheinheimera baltica* that forms blue coloured colonies).

Colonies are circular, smooth, convex, non pigmented and entire. Transparent, turning into slightly opaque with ongoing incubation (>2 wk, 25°C, on half strength marine agar). Cells are Gram-negative, rod-shaped (width 0.6 – 1.2µm, length 0.9 – 2.4µm), oxidase and catalase positive. It reduces nitrate to nitrite. Temperature for growth ranged from 4 to 37°C, with an optimum around 25 °C. NaCl is not needed for growth; growth occurs from 0 to 8% NaCl, with an optimum at 1 to 3%. The strain grows from pH 5.7 to pH 10, with an optimum around neutral pH. The strain hydrolyzes gelatine, lecithin, starch, and tween 80. It does not produce sulfide and does not haemolyse bovine blood. In the API 50CH test system, it produces acid from N-acetyl-glucosamine, amidon, and glycogen; all other API 50CH tests were negative. In the API 20NE test system it shows assimilation of N-acetyl-glucosamine, and as enzymatic activities beta-glucosidase, and protease; in the API ZYM test system alkaline and acidic phosphatase, esterase (C4, C8), leucine arylamidase, trypsine, chymotrypsine, naphtol-phospho-hydrolase, and N-acetyl-beta-glucosaminase were positive. All not mentioned tests of APIZYM and API50CH were negative (for details see Table 1a, IJSEM online). In the Biolog GN2 test system it utilizes as substrates L-alanine, ß-hydroxy-butyric acid, alpha-cyclodextrin, L-alanyl-glycine, and L-threonine (all other Biolog GN2 substrates were negative, for details see Table 1a, IJSEM online). In general, the utilized organic substrates had a very limited spectrum.

Rheinheimera perlucida is of marine or estuarine origin. The G+C content is 48.9 mol%. The type strain is *Rheinheimera perlucida* BA131^{T}. The type strain was deposited in the Deutsche

Sammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) (DSM XXX) and the LMG, Gent (LMG XXX) and the CIP, Paris (CIP XXX).

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REFERENCES

Alavi, M., Miller,T., Erlandson, K., Schneider, R. & Belas, R. (2001). Bacterial community associated with Pfiesteria-like dinoflagellate cultures *Environ. Microbiol.* **3**, 380-396.

Bianchi, A. & Bianchi, M. (1995). Bacterial diversity and ecosystem maintenance: an overview. In: Microbial Diversity and Ecosystem Maintenance, pp. 185-198. Edited by D. L. Hawksworth & R. R. Colwell: CAB International (UNEP).

Brettar, I. & Rheinheimer, G. (1992). Influence of carbon availability on denitrification in the water column of the central Baltic. *Limnol Oceanogr* **37**, 1146-1163.

Brettar, I. & Höfle, M. G. (1993). Nitrous oxide producing heterotrophic bacteria from the water column of the central Baltic: abundance and molecular identification. *Mar Ecol Prog Ser* **94**, 253-265

Brettar, I. , Christen , R. & Höfle, M. G. (2002). *Rheinheimera baltica* gen. nov., sp. nov., a blue coloured bacterium isolated from the central Baltic Sea. *Int. J. Syst. Evol. Microbiol.* **52**, 1851-1857.

Brettar, M. Labrenz, S. Flavier, J. Bötel, H. Kuosa, R. Christen, and M. G. Höfle (2006). Identification of a *Thiomicrospira denitrificans*-like epsilonproteobacterium as a catalyst for autotrophic denitrification in the central Baltic Sea. Appl. Environ. Microbiol. 72: 1364-1372.

Dye, D. W. (1968). A taxonomic study of the genus *Erwinia*. *New Zealand J Sci* **11**, 590-607.

Fonnesbech Vogel, B., Venkateswaran, K., Christensen, H., Falsen, E., Christiansen, G. & Gram, L. (2000). Polyphasic taxonomic approach in the description of *Alishewanella fetalis* gen. nov., sp. nov., isolated from a human foetus. *Int J Syst Evol Bacteriol* **50**, 1133-1142.

Gascuel, O. (1997). BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Molecular Biology and Evolution* 14, 685-695.

Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg N. R. (1994). *Methods for General and Molecular Bacteriology*, American Society for Microbiology, Wash. D.C.

Giovannoni, S. J. & U. Stingl. (2005). Molecular diversity and ecology of microbial plankton. *Nature* **437**, 343-348.

Höfle, M. G. & Brettar, I. (1995). Taxonomic diversity and metabolic activity of microbial communities in the water column of the central Baltic Sea. *Limnol Oceanogr* **40**, 868-874.

Ma, Y., Xue, Y., Grant, W.D., Collins, N. C., Duckworth, A. W., van Steenbergen, R. P. & Jones, B. E. (2004). *'Alkalimonas' amylolytica* gen, nov., sp. nov., and *Alkalimonas delamerensis* gen, nov., sp. nov., novel alkaliphilic bacteria from soda lake lakes in China and East Africa. *Extremophiles* **8**, 193-200.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurements of the G+C content of the deoxyribonucleic acid by high performance liquid chromatography. *Int J Syst Bact* **39**, 159-167.

Moore, E. R. B., Mau, M., Arnscheidt, A., Böttger, E. C., Hutson, R. A., Collins, M. D., Van De Peer, Y., De Wachter, R. & Timmis, K.N. (1996). The determination and comparison of the 16S rRNA gene sequences of species of the genus *Pseudomonas* (sensu strictu) and estimation of the natural intrageneric relationships. *System Appl Microbiol* **19,** 476-492.

Moore, E. R. B., Arnscheidt, A., Krüger, A., Strömpl, C. & Mau, M. (1999). Simplified protocols for the preparation of genomic DNA from bacterial cultures. In *Molecular Microbial Ecology Manual*. 1.6.1: 1-15. Edited by A.D.L. Akkermans, J.D. van Elsas & F.J. de Bruijn, Dordrecht, The Netherlands, Kluwer Academic Press.

Mullis, K. B. & Faloona, E. (1987). Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol* **155,** 335-350.

Oppenheimer, C. H., ZoBell, C. E. (1952). The growth and viability of sixty-three species of marine bacteria as influenced by hydrostatic pressure. *J mar Res* **11**,10-18.

Perrière, G. & Gouy, M. (1996). WWW-Query: An on-line retrieval system for biological sequence banks. *Biochimie* **78,** 364-369.

Pinhassi, J. & Berman, T. (2003). Differential growth response of colony-forming alpha- and gamma-proteobacteria in dilution culture and nutrient addition experiments from Lake Kinneret (Israel), the eastern Mediterranean Sea, and the Gulf of Eilat. *Appl Environ Microbiol.* **69**, 199-211.

Poretsky, R. S., Bano, N., Buchan, A., LeCleir, G., Kleikemper, J., Pickering, M., Pate, W. M., Moran, M. A. & Hollibaugh, J.T. (2005). Analysis of microbial gene transcripts in environmental samples. Appl. Environ. Microbiol. 71, 4121-4126.

Reed, D.W., Fujita,Y. Delwiche, M.E., Blackwelder, D.B, Sheridan, P.P., Uchida,T., & Colwell, F.S. (2002). Microbial communities from methane hydrate-bearing deep marine sediments in a forearc basin. *Appl. Environ. Microbiol.* **68**, 3759-3770.

Romanenko, L.A., Uchino, M., Falsen, E., Zhukove, N.V., Mikhailov, V.V. & Uchimura, T. (2003). *Rheinheimera pacifica* sp. nov., a novel halotolerant bacterium isolated from deep sea water of the Pacific. *Int. J. Syst. Evol. Microbiol.* **53,** 1973-1977.

Sasser, M. (1990). Identification of bacteria by the gas chromatography of cellular fatty acids. *MIDI technical note* **101**. MIDI Inc. Newark, DE, U.S.A.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiology Letters* **25**, 125-128.

FIGURE LEGENDS

Fig. 1. Unrooted phylogenetic tree resulting from the analysis of nearly complete 16S rRNA gene sequences of the most related cultured species plus the closest related environmental sequence (in bold: type strains). The topology shown was obtained using a NJ algorithm and 1000 bootstrap replications. % of bootstraps are indicated only for branches found concomitantly by parsimony (indicated by "+") and maximum likelihood ($P<0.01$) (indicated by "*"), and therefore define robust clusters.

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Fig. 1a. Cells of strain BA131^T as visualized after after Pt/C shadow casting by electron microscopy (A), and by phase contrast microscopy (B). Cells shown were grown in half strength marine broth (Difco), at exponential growth.

1 ***Table 1.*** Differential phenotypic features to distinguish BA131^T from related species

2 (for references see Table 2)

Table 1. Differential phenotypic features of strains related to BA131^T

Species Strain designation	*Rheinheimera baltica* **OSBAC1^{T} *1**	*Rheinheimera pacifica* **KMM 1406^{T} *2**	*Rheinheimera perlucida* **BA131^{T}**	*Alishewanella fetalis* **CCUG 30811^{T} *2,3**	*'Alkalimonas amylolytica'* **N10 *4**	*'Alkalimonas delamerensis'* **1E1 *4**
Site of isolation	Baltic Sea station LL12, 5m	NW Pacific deep sea, 5000m	Baltic Sea station Teili, 5m	Human fetus	Soda lake China	Soda lake East Africa
Character						
Size	0.4-1.5 x 0.9-4.5	0.6-0.8 x 1.8-2.0	0.6-1.2 x 0.9-2.4	0.5-1.0 x 2.0	0.5-0.7 x 2.0-4.0	0.5-0.7 x 1.7-3.3
Motility	1 polar flagellum	many flagella, polar, lateral	1 polar flagellum	no flagellum	1 polar flagellum	1 polar flagellum
Colony colour	blue	no pigment	no pigment	no pigment	no pigment	no pigment
G+C mol%	48.9	49.6	48.9	50.6	52.5	55.4
Growth in NaCl:	0-6%	0-8%	0-8%	3-8%	0-7%	0-7%
Optimum	1-3	nd	1-3	nd	2-3	3
Growth at °C:	4-30°C	4-37°C	4-37°C	25-41°C	10-42°C	10-42°C
Optimum	20-25°C	nd	20-30°C	37°C	37°C	37°C
Growth at pH	pH 5.7-10	nd	pH 5.7-10	nd	pH 7.5-11	pH 8-11
Optimum	7	nd	7	nd	10	10
Hydrolysis of:						
Starch	+	+	+	-	+	+
Use of electron acceptors:						
Thiosulphate	-	-	-	+	nd	nd
TMAO	-	nd	-	+	nd	nd
NO_3 to NO_2	-	-	+	+	+	+
NO_3 to N_2	-	-	-	+	nd	nd
Acid production from:						
D-Glucose	+	-	-	-	-	-
Cellobiose	+	nd	-	nd	-	-
Maltose	+	nd	-	-	-	-
Beta-gentiobiose	+	nd	-	nd	nd	nd
Enzymatic activity:						
Esterase (C4)	-	+	+	nd	nd	nd
Chymotrypsin	-	+	+	nd	nd	nd
Phosphat., acid.	-	-	+	nd	nd	nd
N-ac.-ß-glucosamin.	-	+	+	nd	nd	nd
Assimilation of:						
Arabinose	-	+	-	-	nd	nd
N-acetyl-glucosamine	+	+	+	-	nd	nd
Citrate	-	+	-	-	nd	nd
Glucose	+	-	-	-	nd	nd
Maltose	+	+	-	-	nd	nd
Utilization of:						
Alpha-cyclodextrin	-	nd	w	nd	nd	nd
Tween 40	+	nd	-	nd	nd	nd
Beta-hydro-butyric acid	-	nd	+	nd	nd	nd
p-Hydro-phenylacetate	+	nd	-	nd	nd	nd
L-Alanine	-	nd	+	nd	+	+
L-Alanyl-glycine	-	nd	w	nd	nd	nd
L-Threonine	-	nd	w	nd	nd	nd
Glucose	-	nd	-	nd	+	+
Mannose	-	nd	-	nd	+	+
Maltose	-	nd	-	nd	+	+
Trehalose	-	nd	-	nd	+	+
Cellobiose	-	nd	-	nd	+	+
Pyruvate	-	nd	-	nd	+	+
Serine	-	nd	-	nd	+	+
Proline	-	nd	-	nd	+	+
Asparagine	-	+	-	nd	+	+
Arginine	-	+	-	nd	+	+
Lysine	-	+	-	nd	+	+
Glutamine	-	nd	-	nd	+	+
Sucrose	-	nd	-	nd	+	-
Rhamnose	-	nd	-	nd	+	-
Fructose	-	nd	-	nd	-	+
Acetic acid	-	+	-	nd	-	o
Glycerol	-	+	-	nd	nd	nd

Legend:
response: +, positive; w, weak; -, negative; nd, no data available.

Table 2. Fatty acid composition of BA131^T in comparison with related species

Fatty acid/strain (fatty acids are listed only when exceeding 0.5% for at least one of the strains, in bold: >5%)

Species Strain designation	*Rheinheimera pacifica* **KMM 1406^T** [*2]	*Rheinheimera baltica* **OSBAC1^T** [*1]	*Rheinheimera baltica* **OSBAC1^T** [*1]	*Rheinheimera perlucida* **BA131^T**	*Rheinheimera perlucida* **BA131^T**	*Alishewanella fetalis* **CCUG 30811^T** [*3]	*'Alkalimonas amylolytica'* **N10** [*4]	*'Alkalimonas delamerensis'* **1E1** [*4]
Growth conditions	Marine Agar, 28°C	1/2 Marine agar, 28°C	Blood agar, 30°C	1/2 Marine agar, 28°C	Blood agar, 30°C	Blood agar, 30°C	Glucose-Yeast-Pepton-medium, pH 9.5, 37°C	Glucose-Yeast-Pepton-medium, pH 9.5, 37°C
10:0					0.13	0.5		
11:0			0.16	0.25	0.51	1.5		
11:0 3-OH			2.49	1.15	1.78	2.5		
12:0		1.43	1.21	1.08	1.28	0.8	0.32	0.32
12:0 3-OH		**5.27**	**5.44**	3.13	3.27	2.0	1.55	3.01
13:0		0.60	0.85	0.43	0.54	0.6	0.28	
13:0 3-OH		1.01	1.72	0.72	1.32	2.7		
14:0		2.28	4.68	1.81	1.13	0.9	1.27	0.65
14:0 3-OH		1.63	1.39	0.49	0.68	1.9		
15:0	2.4	1.72		3.03		**6.7**	4.29	0.28
15:1 omega8c	3.3	1.67	**5.75**	1.97	3.04	4.9	1.47	0.25
15:1 omega6c			0.66	0.30	0.57	0.9	0.17	
16:0 iso	3.7	0.46	0.94	1.20	1.23	1.8		
16:0	**19.1**	**19.53**	**25.23**	**17.11**	**18.24**	**8.9**	**18.91**	**18.08**
16:1 omega9c	25.5	1.72	1.05		0.47			
16:1 omega7c		**33.56**	**33.14**	**33.37**	**23.61**	**19.0**	**14.31**	**8.21**
17:0 iso			0.24		0.47	0.4	0.8	0.45
17:0 anteiso		0.36	0.43	0.52	0.81	0.9	1.75	1.26
17:0	8.1	3.29	2.52	3.36	**7.88**	**10.3**	**6.99**	1.94
17:1 omega8c	**11.7**	**5.68**	**3.07**	**13.87**	**18.3**	**19.5**	**6.94**	1.98
17:1 omega6c		1.02	0.41	0.90	0.88	1.4	0.89	0.33
18:0		0.50	0.45	0.39	0.73		0.77	4.81
18:0 iso	1.3				0.29		0.69	0.56
11meth.18:1 omega7c					0.51		0.51	0.91
18:1 omega7c	**15.7**	**15.87**	3.79	**12.39**	**9.39**	**7.2**	**27.87**	**47.72**
20:1omega7c							0.3	1.92

Legend:

[*1], data from Brettar et al. 2002

[*2] ,data from Romanenko et al. 2003

[*3], data from Fonnesbech Vogel et al. 2000

[*4], data from Ma et al. 2004

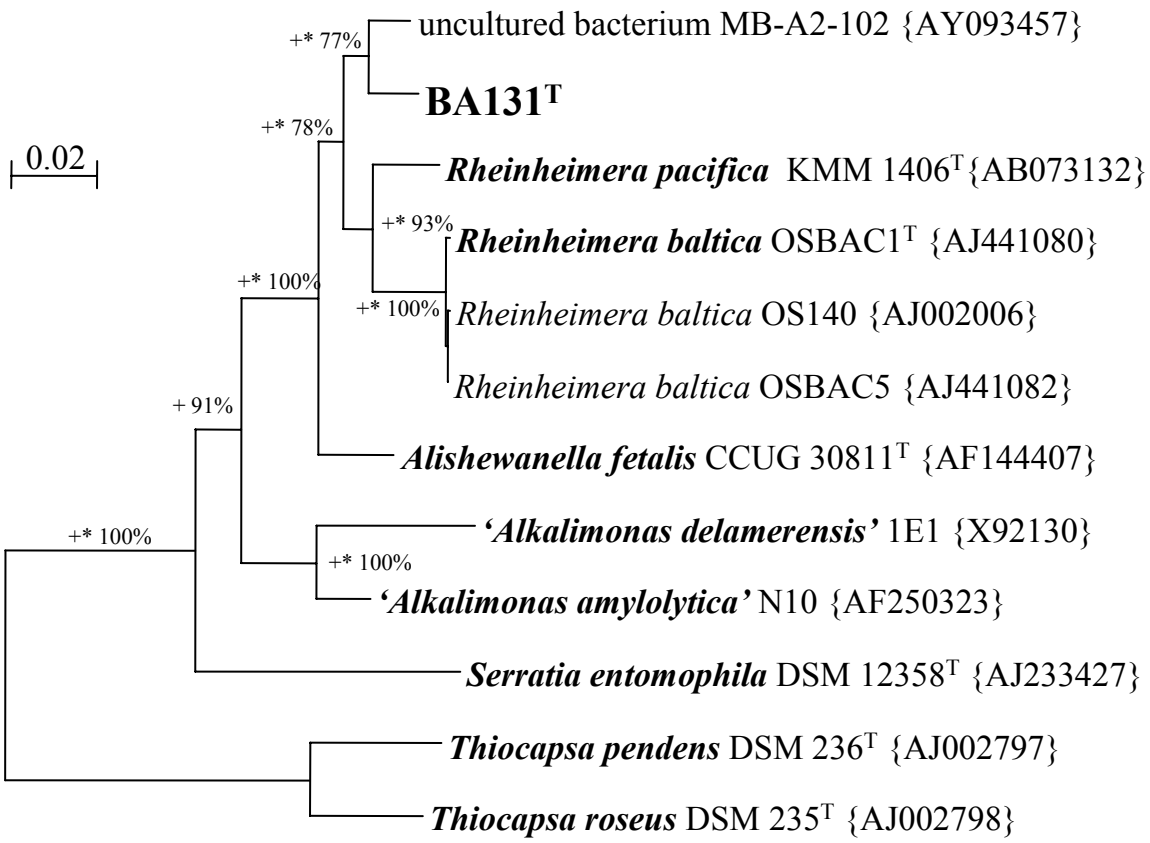
uncultured bacterium MB-A2-102 {AY093457}
+* 77%
BA131T
+* 78%
0.02
Rheinheimera pacifica KMM 1406T{AB073132}
+* 93%
Rheinheimera baltica OSBAC1T {AJ441080}
+* 100%
+* 100%
Rheinheimera baltica OS140 {AJ002006}
Rheinheimera baltica OSBAC5 {AJ441082}
+ 91%
Alishewanella fetalis CCUG 30811T {AF144407}
+* 100%
'Alkalimonas delamerensis' 1E1 {X92130}
+* 100%
'Alkalimonas amylolytica' N10 {AF250323}
Serratia entomophila DSM 12358T {AJ233427}
Thiocapsa pendens DSM 236T {AJ002797}
Thiocapsa roseus DSM 235T {AJ002798}

Fig. 1

IJSEM online: ***Table 1a.*** **Positive results obtained by API and BIOLOG tests and tests for substrate hydrolysis for BA131^T**

Substrate	**API50CH (acid production)**
N-Acetylglucosamine	+
Amidon	+
Glycogen	+

all other tests of the API50CH test had negative results, i.e:
Glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, beta-methyl-D-xylosid, galactose, D-glucose, D-fructose, D-mannose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, alpha-methyl-D-mannosid, alpha-methyl-D-glucosid, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, saccharose, trehalose, inulin, melezitose, D-raffinose, xylitol, beta-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconat, 2-ketogluconat, 5-ketogluconat

Activity	**API 20NE**
NO_3^- -> NO_2^-	+
Beta-glucosidase	+
Protease	+
N-acetyl-glucosamine assimilation	+

all other tests of the API20NE test had negative results, i.e:
NO_3^-reduction to N_2, trypton/indole, glucose fermentation, arginine-dihydrogenase, urease, beta-galactosidase, glucose assimilation, arabinose assimilation, mannose assimilation, mannit assimilation, maltose assimilation, gluconate assimilation, caprat assimilation, adipat assimilation, malate assimilation, citrate assimilation, phenylacetate assimilation

Activity	**API ZYM**
Phosphatase, alkaline	+
Esterase (C4)	+
Esterase, lipase (C8)	+
Leucine-arylamidase	+
Trypsine	+
Chymotrypsine	+
Phosphatase, acidic	+
Naphtol-phospho-hydrolase	+
N-acetyl-beta-glucosaminase	+

all other tests of the APIZYM test had negative results, i.e:
Lipase (C14), valin-arylamidase, cystin-arylamidase, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, alpha-mannosidase, alpha-fucosidase

Utilization of	**BIOLOG GN2**
Alpha-cyclo-dextrin	**w**
L-Alanine	+
L-Alanyl-glycine	**w**
Beta-hydro-butyric acid	+
L-Threonine	**w**

all other tests of the BIOLOG GN2 test had negative results, i.e:
Dextrin, glycogen, tween 40, tween 80, N-acetyl-D-galactosamin, N-acetyl-D-glucosamin, adonitol, L-arabinose, D-arabitol, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose,alpha-D-glucose, m-inositol, alpha-D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, beta-methyl D-glycoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, methylpyruvate, mono-methyl succinat, acetic acid, cis-aconitic acid, citric acid, formic acid, D-galactic acid, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, alpha-hydro-butyric acid, gamma-hydro-butyric acid, p-hydro-phenylacetic acid, itaconic acid, alpha-keto butyric acid, alpha-keto glutaric acid, alpha-keto valeric acid, D,L-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromo succinic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, hydroxy L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, D,L-carnitine, gamma-amino butyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-amino ethanol, 2,3-butanediol, glycerol, D,L-alpha-glcerolphosphate, glucose-1-phospate, glucose-6-phosphate

Hydrolysis of	**(Gerhardt et al. 1994)**
Starch	+
Gelatin	+
Tween 80	**w**
Lecithine	+

response: +, good; w, weak

Figure 1a, IJSEM online-material:

Fig. 1a. Cells of strain BA131T as visualized after after Pt/C shadow casting by electron microscopy (A), and by phase contrast microscopy (B). Cells shown were grown in half strength marine broth (Difco), at exponential growth.