***Carnobacterium inhibens* sp. nov., isolated from the intestine of Atlantic salmon (*Salmo salar*)**

Anna Jöborn,¹ Matthias Dorsch,² J. Christer Olsson,¹ Allan Westerdahl¹ and Staffan Kjelleberg²

Author for correspondence: Anna Jöborn. Tel: +46 31 7732599. Fax: +46 31 7732599.
e-mail: anna.joborn@o.lst.se

¹ Department of General and Marine Microbiology, University of Göteborg, Medicinaregatan 9C, 413 90 Göteborg, Sweden

² School of Microbiology and Immunology, University of New South Wales, Sydney, NSW 2052, Australia

Strain K1^T, isolated from the gastrointestinal tract of Atlantic salmon (*Salmo salar*), has the capacity to inhibit the growth of the fish pathogens *Vibrio anguillarum* and *Aeromonas salmonicida*. Strain K1^T is a motile Gram-positive psychrophilic rod that lacks both catalase and oxidase, which does not grow on acetate containing media, but grows at pH 9 and in TSB with up to 6 % sodium chloride content. Strain K1^T is facultatively anaerobic and tryptone as a sole source of nutrient promotes growth. The most abundant cellular fatty acid of strain K1^T is oleic acid (18:1*cis*9). Based on 16S rDNA sequence comparisons, it is suggested that strain K1^T is phylogenetically closely related to *C. alterfunditum*. However, the unique phenotypic attributes of strain K1^T suggest that it represents a new species. The name *Carnobacterium inhibens* is proposed, for which the type strain is K1^T (= CCUG 31728^T).

Keywords: *Carnobacterium inhibens*, *Vibrio anguillarum*, *Aeromonas salmonicida*, intestinal microbiota, Atlantic salmon

INTRODUCTION

The gastrointestinal tract of fish is a nutrient rich habitat suitable for microbial growth. The information on composition and abundance of bacteria in the gastrointestinal tract of fish is steadily increasing. However, there are few examples where a specific function can be assigned to a certain bacterial group or species. It is recognized that some isolated bacteria from the gastrointestinal tract of fish have the ability to inhibit growth of bacterial fish pathogens *in vitro* (Schröder *et al.*, 1980; Strøm, 1988; Westerdahl *et al.*, 1991). It can be hypothesized that these inhibitory bacteria may be able to prevent the colonization and proliferation of bacterial pathogens that invade the gastrointestinal tract of fish. A group of *Lactobacillus*-like bacteria were isolated during a screening procedure (Jöborn *et al.*, 1997) designed to select intestinal bacteria in Atlantic salmon (*Salmo salar*) with capacity to prevent the growth of the two common bacterial fish pathogens *Vibrio anguillarum* and *Aeromonas salmonicida*. There exist a number of reports in the literature on the presence of atypical lactic acid bacteria in the

intestine of fish (Baya *et al.*, 1991; Evelyn & McDermott, 1961; Humphrey *et al.*, 1987; Knøchel, 1981; Kvasnikov *et al.*, 1977; Ringø, 1993; Ross & Toth, 1974; Schröder *et al.*, 1980). However, the phylogeny of this group of bacteria needs to be investigated in more detail in order to arrive at a better understanding of their appropriate taxonomic assignments as well as their role in the gastrointestinal tract of fish.

In this study, the phylogeny of one of the *Lactobacillus*-like bacteria isolated from Atlantic salmon, designated strain K1^T, with marked growth inhibitory effects *in vitro* against *Vibrio anguillarum* and *Aeromonas salmonicida*, is evaluated. The inhibitory activity and colonization potential of strain K1^T have previously been investigated (Jöborn *et al.*, 1997). In this paper, strain K1^T is analysed by 16S rDNA sequence comparison and characterized by a range of biochemical and physiological tests.

METHODS

Media, diluent and culture conditions. The media and diluent used were: tryptic soy broth (TSB; Difco) and TSBS (TSB supplemented with 2 % NaCl); TSA (TSB + 15 % agar) and TSAS (TSA supplemented with 2 % NaCl); TSAS soft agar

The EMBL accession number for the 16S rDNA sequence of *C. inhibens* strain K1^T is Z73313.

(TSBS + 5% agar); marine agar (MA; Difco); nutrient agar (NA; Difco); brain heart infusion broth (BHI; Difco); Lactobacilli MRS (Difco); Cholera medium (TCBS; Oxoid); tryptone broth (TB) and tryptone agar (TA, 4% tryptone (Difco) + 15% agar); marine minimal medium (MMM; Östling *et al.*, 1991); NSS (Mårdén *et al.*, 1985); Horse-blood agar (HBA, i.e. TSA supplemented with 5% horse blood); Columbia agar with 5% horse blood (CAHB).

Bacteria. An atypical lactic acid bacteria, denoted strain K1^T, was found during a screening of bacteria, from the intestine of Atlantic salmon (*Salmo salar*) (Jöborn *et al.*, 1997), which excrete substances which inhibit the growth of *Vibrio anguillarum* and *Aeromonas salmonicida*. The following organisms (EMBL accession numbers are in parentheses) were included in the construction of a phylogenetic tree: *Carnobacterium alterfunditum* (L08623), *Carnobacterium divergens* (X54270), *Carnobacterium funditum* (S86170), *Carnobacterium gallinarum* (X54269), *Carnobacterium mobile* (X54271), *Carnobacterium piscicola* (X54268), *Vagococcus fluvialis* (X54258) and *Vagococcus salmoninarum* (X54272). Also in this study *Carnobacterium alterfunditum* (CCUG 34643), *C. divergens* (CCUG 30094), *C. gallinarum* (CCUG 30095), *C. mobile* (CCUG 30096), *Vibrio anguillarum* HI11360 and *A. salmonicida* ATCC 14174 were used to test growth inhibition.

Morphological and biochemical characterization of strain K1^T. The following conventional methods were employed to characterize strain K1^T: visual inspection of colony morphology and pigmentation on TSBS plates; cell morphology and motility from TSBS cultures was examined by light microscopy ($\times 1250$ magnification); Gram staining and staining of the flagella (Heimbrook *et al.*, 1989) was performed on overnight cultures in TSBS; spore staining was performed on stationary-phase cultures with malachite-green and viability test of a culture heated for 10 and 30 min at 65 and 100 °C; haemolysis was tested on HBA; presence of catalase and oxidase was tested with cells cultivated on TSBS. Since some lactic acid bacteria produce a pseudo-catalase when supplied with haemoglobin (Axelsson, 1993), cells were grown on HBA and subsequently tested for catalase activity.

The utilization of a number of carbon sources and activity of various enzymes were tested at the National Collection of Industrial and Marine Bacteria, limited, Great Britain (NCIMB) and the Culture Collection, University of Göteborg, Sweden (CCUG). The results were complemented with tests using the API rapid ID32 STREP (bioMérieux) and API 20 (API Systems).

Growth characterization of strain K1^T. The growth of strain K1^T was monitored by optical density at 600 nm at 23 °C in aerobic TSBS cultures and the pH change during growth was followed. Furthermore, the ability of strain K1^T to grow at 0, 15, 28, 30 and 37 °C was examined on TSAS plates and in TSBS. The growth in TSBS at various pH was tested. The growth in TSB and TB supplemented with NaCl was monitored in the range of 0–10% NaCl. Acid production after growth on the marine oxidation-fermentation medium (MOF) (Leifson, 1963) was tested. The ability of strain K1^T to grow anaerobically at 23 °C was tested using TSAS plates in an anaerobic box (Forma Scientific). Growth was also tested in MMM (glucose excluded) to which had been added sucrose, maltose, mannitol, trehalose, Casamino acids, peptone, tryptone and yeast extract at various concentrations and in various combinations. Growth on different solid media including NA, TSA, TSAS, BHI, MA, MMM, TCBS, MRS, HBA and TA was monitored.

Cellular fatty acid analysis. The cellular fatty acid composition was analysed by staff at the Culture Collection, University of Göteborg, Sweden (CCUG). The bacterium was grown on CAHB for 24 h at 30 °C. The cells were harvested, washed in distilled water, and saponified. The liberated cellular fatty acids were methylated and extracted. The resulting cellular fatty acid methyl esters were analysed by a system similar to the MIDI-system (MIDI) on a Hewlett Packard 5890 gas chromatograph with an auto sampler (HP 7673). The cellular fatty acid methyl esters were separated by GC using a HP-5 cross-linked 5% Ph Me (5% diphenyl- and 95% dimethyl-polysiloxane) silicone column (25 m $\times$ 0.2 mm $\times$ 0.33 μ m). The identification of peaks were done according to a Hewlett Packard standard.

Antimicrobial susceptibility. The antimicrobial susceptibility of strain K1^T was determined using antibiotic-impregnated filter discs (Oxoid). The filter discs were placed on the surface of TSAS plates, that previously had been seeded with strain K1^T. The plates were incubated at 23 °C for 48 h and thereafter examined for zones of no growth around the filter discs. The following antibiotics were tested at various concentrations: gentamicin (30 μ g), neomycin (30 μ g), erythromycin (15 μ g), rifampicin (5 μ g), tetracycline (30 μ g), ampicillin (10 μ g), kanamycin (30 μ g) and nalidixic acid (30 μ g).

Isolation of genomic DNA. Cells were grown on TSAS plates for 24 hours at 23 °C. Cells from one plate were overlaid with 5 ml TE buffer (Maniatis *et al.*, 1982) and suspended in the buffer using a sterile glass spatula. The cell suspension was transferred into Eppendorph tubes and the DNA was isolated and purified as described previously (Rainey *et al.*, 1992).

Amplification and sequencing of 16S rDNA. 16S rDNA was PCR-amplified as published previously (Dorsch & Stackebrandt, 1992) and the products purified employing the Prep-A-Gene kit (Bio-Rad) according to the manufacturer's instructions. Purified PCR products were sequenced using a modified protocol for the Sequenase kit version 2.0 (USB) as published previously (Dorsch & Stackebrandt, 1992). Sequencing products were separated on wedge-shaped gels (8% polyacrylamide, 8 M urea) in Macrophore Systems (LKB-Pharmacia). Electrophoresis was performed for 2.5 and 6 h (2200 V, constant voltage). Gels were washed in 10% acetic acid, 10% acetic acid/10% methanol (20 min each) and dried at 80 °C for 2 h. X-ray films (Amersham) were exposed for 12–18 h. The generated sequence covered positions 11–1512 according to the *Escherichia coli* numbering system (Brosius *et al.*, 1978).

Phylogenetic analysis based on 16S rDNA. The 16S rDNA sequence was subjected to 'Fast Analysis' against the GenBank database through ANGIS (Australian National Genomic Information Service) in order to determine the organisms exhibiting the highest 16S rDNA sequence homologies. The following organisms (EMBL accession numbers are in parentheses) were included in the construction of a phylogenetic tree to determine the phylogenetic position of the strain K1^T: *C. alterfunditum* (L08623), *C. divergens* (X54270), *C. funditum* (S86170), *C. gallinarum* (X54269), *C. mobile* (X54271), *C. piscicola* (X54268), *Vagococcus fluvialis* (X54258) and *Vagococcus salmoninarum* (X54272). A multiple sequence alignment was created using CLUSTAL W (version 1.5) and a phylogenetic tree constructed employing the neighbour-joining method of Saitou & Nei (1987). Both programs were accessed through ANGIS. Sequence gaps were not included in the phylogenetic

analysis. Due to partially incomplete sequences of the included reference organisms, the alignment covered positions 107–1471 (*E. coli* numbering system).

Growth inhibition by *Carnobacterium* species. The ability of the *Carnobacterium* species *C. alterfunditum*, *C. divergens*, *C. gallinarum* and *C. mobile* to inhibit the growth of *Vibrio anguillarum* and *A. salmonicida*, as well as strain K1^T, was tested by a double agar technique (Westerdahl *et al.*, 1991). The capability of strain K1^T to inhibit the *Carnobacterium* species was also tested.

RESULTS

Morphological, biochemical and growth characteristics of strain K1^T

Strain K1^T is a Gram-positive, asporogenous rod. The colonies appear as buff-coloured and semi-translucent when grown under anaerobic conditions and whitish when grown aerobically. The colonies are 1–2 mm in diameter, raised, entire and circular. The morphology of strain K1^T in the exponential growth phase in TSBS is as 0.2 × 0.3–0.7 µm single or double rods (often occurring as V-forms). In the stationary phase, the cells are 0.2 × 0.5–1.2 µm in pairs or as small chains of four cells. The cells are motile, with a single subpolar flagellum.

Strain K1^T grows on all solid media tested except TCBS and MRS. It is fermentative in Leifson media (weak acid but no gas). Under anaerobic growth conditions the strain K1^T culture reaches a higher cell density than in aerated cultures. It grows at a temperature range between 0 and 30 °C, at salinities between 0% and 6% and at a pH range of 5.5–9.0. Strain K1^T produces α-haemolysis on HBA.

Strain K1^T grows (and produces growth inhibitors) in 3% tryptone in MMM, as a sole source of nutrients. Casamino acids, yeast extract, pepton or various sugars, respectively, did not result in growth at the concentrations tested. However, various combinations of the nutrients did support growth. The generation time of the strain K1^T culture in TSBS at 23 °C was 1.5 ± 0.1 h, as determined by optical density at 600 nm. At the growth conditions employed in this study, the culture entered the stationary growth phase by a sharp transition at an optical density of approximately 0.25. When the growth had ceased the pH had decreased from 7.2 to 6.9. Other phenotypic attributes of strain K1^T are presented together with literature data about *C. funditum*, *C. alterfunditum* and *C. piscicola* in Table 1. The analysis were performed by CCUG, NCIMB and by using API rapid ID32 STREP and API 20.

Strain K1^T did not produce acetoin and was not able to reduce nitrate to nitrite. Strain K1^T was found to hydrolyse hippurate. β-Glucosidase activity was demonstrated whereas no activity of the following enzymes could be detected: urease, β-galactosidase, β-glucuronidase, α-galactosidase, arginine dihydrolase, alanylphenylalanyl-proline arylamidase, acid pyroglutamine arylamidase, N-acetyl-β-glucosaminidase.

The most abundant cellular fatty acids of strain K1^T, according to the CCUG analysis, were found to be 16:0 (31.1%), 16:1 (24.2%) and 18:1_{cis}9 (23.4%). Other fatty acids found were 18:2_{cis}9,12 or 18:0 (10.8%), 14:0 (5.4%) and 18:0 (3.5%). A comparison of the fatty acid composition of strain K1^T with various closely related *Carnobacterium* species is provided in Table 2.

Strain K1^T was sensitive to gentamicin (30 µg), neomycin (30 µg), erythromycin (15 µg), rifampicin (5 µg), tetracycline (30 µg), ampicillin (10 µg) and kanamycin (30 µg), whereas it was resistant to nalidixic acid (30 µg).

Inhibitor production within the genus *Carnobacterium*

The growth inhibition caused by a number of *Carnobacterium* species was investigated. *C. alterfunditum*, *C. mobile*, *C. divergens* and *C. gallinarum* did not inhibit *Vibrio anguillarum* HI11360 or *Aeromonas salmonicida* ATCC 14174, but they inhibited the growth of strain K1^T. As previously shown (Jöborn *et al.*, 1997), strain K1^T suppresses the growth of *Vibrio anguillarum* and *A. salmonicida*. However, strain K1^T did not inhibit the growth of the different *Carnobacterium* species tested.

A phylogenetic tree based on 16S rDNA sequence comparisons

An unrooted phylogenetic tree was constructed (Fig. 1). Strain K1^T showed close relatedness to *C. alterfunditum*. The phylogenetic groupings of *C. gallinarum*/*C. piscicola*, *C. mobile*/*C. funditum* and the isolated position of *C. divergens* are in accordance with previously published data (Franzmann *et al.*, 1991; Wallbanks *et al.*, 1990). *Vagococcus salmoninarum* and *Vagococcus fluvialis* were included as representatives of a remotely related genus. The significance of the branching points in the tree as determined by bootstrapping (Saitou & Nei, 1987) was 73% (*C. funditum*/*C. mobile*), 98.1% (*C. alterfunditum*/strain K1^T), 100% (*C. gallinarum*/*C. piscicola*) and 100% (*Vagococcus salmoninarum*/*Vagococcus fluvialis*).

DISCUSSION

In the present study we examined the biochemical and genetic characteristics of a non-aciduric lactic acid bacterium isolated from the gastrointestinal tract of Atlantic salmon. It possesses the key attributes that confine the genus *Carnobacterium* (Collins *et al.*, 1987). Strain K1^T is non spore-forming and cells occur as straight rods (singly, as pairs or as short chains). It is motile, Gram-positive, facultatively anaerobic and heterofermentative. It grows at 10 °C but not at 35 °C. It does not grow in 8% NaCl or in acetate containing media. It is catalase-negative and is not able to reduce nitrate to nitrite. The major fatty acids are of straight-

Table 1. Physiological and biochemical characteristics of *C. inhibens* sp. nov. (strain K1^T) and *C. funditum* (DSM 5972^T; Franzmann *et al.*, 1991), *C. alterfunditum* (DSM 5970^T; Franzmann *et al.*, 1991), *C. piscicola* (DSM 20730^T; Hiu *et al.*, 1984)

All strains were negative for the following attributes: adonitol, rhamnose, dulcitol, inositol, sorbitol, starch, glycogen and nitrate reduction.

Attribute	<i>C. inhibens</i> K1 ^T	<i>C. funditum</i> DSM 5972 ^T	<i>C. alterfunditum</i> DSM 5970 ^T	<i>C. piscicola</i> DSM 20730 ^T
Motile	+	+	+	—
Rods	+	+	+	+
Spores	—*†	—	—	—
Catalase	—‡	—	—	—
Pseudo-catalase	+§	NT	NT	NT
Oxidase	—‡	—	—	—
Dextrin	—	NT	NT	—
Glycerol	—	W	W	+
Erythritol	—	NT	NT	—
Arabinose	+(L)	—	—	—
Ribose	+	—	—	+
Xylose	—(D and L)	—	—	—(D and L)
Methyl xyloside	—	NT	NT	—
Galactose	+	W	W	+
Glucose	+(D)	+	+	+(D)
Fructose	+(D)	+	W	+(D)
Mannose	+(D)	+	W	+(D)
Sorbose	—(L)	NT	NT	—(L)
Mannitol	+	+	—	+
Methyl D-mannoside	—	NT	NT	—
Methyl D-glucoside	—	NT	NT	W
N-Acetylglucosamine	+	NT	NT	+
Amygdalin	+	—	+	+
Arbutin	+	NT	NT	+
Aesculin	+	—	—	+
Salicin	+	+	NT	+
Cellobiose	+	+	W	+(D)
Maltose	+	+	W	+
Lactose	W	—	—	—
Melibiose	—	—	—	W
Sucrose	+	+	W	+
Trehalose	+	+	—	+(D)
Inulin	W	—	—	—
Melezitose	—	—	—	—(D)
Raffinose	—(D)	—	—	W(D)
Xylitol	—	NT	NT	NT
Gentibiose	+	NT	NT	NT
Turanose	—(D)	NT	NT	NT
Lyxose	—(D)	NT	NT	NT
Tagatose	—(D)	NT	NT	NT
Fucose	—(D and L)	NT	NT	NT
Arabitol	—(D and L)	NT	NT	NT
Gluconate	—	NT	NT	NT
2-Keto gluconate	—	NT	NT	NT
5-Keto gluconate	—	NT	NT	NT
Growth at (°C):				
0	+*	+	+	—
30	+*	—	—	+
35	—*	—	—	+
40	—*	—	—	+

Table 1 (cont.)

Attribute	<i>C. inhibens</i> K1 ^T	<i>C. funditum</i> DSM 5972 ^T	<i>C. alterfunditum</i> DSM 5970 ^T	<i>C. piscicola</i> DSM 20730 ^T
Urease	—	NT	NT	—
H ₂ S production	—	NT	NT	—
Gelatin hydrolysis	—	—	—	NT
Gas from glucose	—	NT	NT	—

* Test performed in TSBS.

† Test performed in MMM.

‡ Test performed on TSA.

§ Test performed on HBA.

Table 2. Percentages (w/w) of total fatty acids from *C. inhibens* sp. nov. compared to data in the literature for a number of *Carnobacterium* species

Bacterial species	14:0	16:1 <i>cis</i> 7	16:1 <i>cis</i> 9	16:1	16:0	18:2 <i>cis</i> 9,10	18:2 <i>cis</i> 9,12	18:1	18:1 <i>cis</i> 9	18:0	cyclo- 19 <i>cis</i> 9	20:1 <i>cis</i> 11
<i>C. inhibens</i> sp. nov.	5.4			24.2	31.1		10.8*		23.4	3.5		
<i>C. alterifunditum</i> pf1†	5.9	21.8			33.2	0			18.9	4.1		1.6
<i>C. mobile</i> MT37‡	2.0		25.5	2.5	16.0			1.5	48.5	2.5		
<i>C. funditum</i> pf2†	3.1	16.5			32.2	0.7			20.7	5.0		0.6
<i>C. divergens</i> NCDO 2763‡	10.5		11.5	1.5	20.0			2.0	28.0	4.0	19.5	
<i>C. piscicola</i> NCDO 2762‡	10.0		11.0	2.0	17.5			0.5	51.5	3.5		

* Could be 18:0 instead.

† Franzmann *et al.* (1991).‡ Collins *et al.* (1987).

chain saturated and monounsaturated types. The C18:1 compound is a *cis*9,12 isomer.

Members of the genus *Carnobacterium* have been isolated from a wide range of environments such as refrigerated meat products (Montel *et al.*, 1991), salmonid fish (Hiu *et al.*, 1984) and anoxic lake water in Antarctica (Schröder *et al.*, 1980). They are difficult to distinguish from *Lactobacillus* sp. given the many characteristics representatives of these genera have in common. Both these bacterial groups are non-sporogenous Gram-positive rods that lack catalase and oxidase, and produce lactic acid during sugar fermentation. Carnobacteria are able to proliferate at low temperatures (psychrophily), but this is also a known feature for some *Lactobacillus* strains (Schröder *et al.*, 1980). However, nearly all carnobacteria fail to grow on acetate containing media, while most lactobacilli do grow on such media. Moreover, carnobacteria are able to grow at a more alkaline pH than lactobacilli and some carnobacteria are motile while the lactobacilli are not. Another way to distinguish carnobacteria from lactobacilli is to compare the composition of the cellular fatty acids. Carnobacteria contain oleic acid as one of the major fatty acids (Collins *et al.*, 1987) while lactobacilli do not. By comparing the 16S rDNA

sequences it has been concluded that the current species in the genus *Carnobacterium* are more closely related to the genus *Vagococcus* than to *Lactobacillus* (Wallbanks *et al.*, 1990).

The 16S rDNA sequence of strain K1^T strongly suggests it to be classified as a *Carnobacterium* species (Fig. 1) (this conclusion is in accordance with a previous sequence analysis, data not shown). Strain K1^T has some unique characteristics previously not described for members of the genus *Carnobacterium*. Firstly, it produces growth inhibitors *in vitro* (Jöborn *et al.*, 1997) that suppress the growth of the bacterial fish pathogens *Vibrio anguillarum* and *A. salmonicida*, a property not found in the other *Carnobacterium* species tested (this study). Secondly, the fatty acid profile of strain K1^T does not correspond to any known profile within the genus *Carnobacterium* (Table 2). Finally, the phenotypic attributes of strain K1^T (Table 1) differ from the characteristics of other *Carnobacterium* species.

A well-known bacterial fish pathogen *Carnobacterium piscicola* (Hiu *et al.*, 1984) has previously been isolated from salmonid fish. The sequence analysis shows that *C. piscicola* and strain K1^T belong to different groups

Fig. 1. Unrooted phylogenetic tree based on the comparison of the 16S rDNA sequence of the *Carnobacterium inhibens* sp. nov. with 16S rDNA sequences of *C. alterfunditum* (L08623), *C. divergens* (X54270), *C. funditum* (S86170), *C. gallinarum* (X54269), *C. mobile* (X54271), *C. piscicola* (X54268), *Vagococcus fluvialis* (X54258) and *Vagococcus salmoninarum* (X54272). Bar, 1% sequence divergence.

within the genus *Carnobacterium* (Fig. 1). Moreover, strain K1^T differs from the *C. piscicola* (type strain) concerning utilization of seven carbon sources, the motility and the temperature range supporting growth (Table 1). Furthermore, strain K1^T was not able to cause disease in a previously performed infection study in Atlantic salmon (Jöborn *et al.*, 1997), indicating it to be non-virulent.

There are three motile *Carnobacterium* species described (Collins *et al.*, 1987; Franzmann *et al.*, 1991; Spielmeyer *et al.*, 1993). The phenotypic attributes of *C. mobile*, *C. alterfunditum* and *C. funditum* show many similarities with strain K1^T (see Table 1; Collins *et al.*, 1987). They all form motile rods, and are heterofermentative, catalase-negative and oxidase-negative bacteria which grow at high salinities. The 16S rDNA sequence similarity ranking shows that *C. alterfunditum* and strain K1^T form one group, and *C. funditum* and *C. mobile* cluster together in another group (Table 1). However, the sequence variation among the current species in the genus *Carnobacterium* is low (Wallbanks *et al.*, 1990) making it hard to distinguish between species solely based on sequence analysis.

The biochemical analysis confirms that the motile *Carnobacterium* species differ from strain K1^T. *C. mobile* diverges from strain K1^T concerning the utilization of at least five carbon sources, it grows at 35 °C and the percentage of the fatty acid 18:1*cis*9 is more than twice as high in *C. mobile* compared to strain K1^T (Collins *et al.*, 1987). *C. funditum* and *C. alterfunditum* differ from strain K1^T regarding temperature sup-

porting growth. Moreover, *C. funditum* differs from strain K1^T concerning its ability to utilize glycerol, arabinose, ribose, galactose, amygdalin, aesculin, lactose and inulin and *C. alterfunditum* concerning its ability to utilize glycerol, arabinose, ribose, galactose, fructose, mannose, mannitol, aesculin, cellobiose, maltose, lactose, melibiose, trehalose and inulin. Moreover, strain K1^T is able to hydrolyse hippurate, which is not the case for the other two *Carnobacterium* species.

The cellular fatty acid profiles for *C. funditum* and *C. alterfunditum* show similarities to strain K1^T in respect to all but two fatty acids. Strain K1^T contains 18:2*cis*9,12 (10.8%) which has not been identified in the other two strains (Table 2). It also contains 16:1 (24.2%), but the exact identity of this fatty acid peak is not clarified. *C. funditum* and *C. alterfunditum* contain 16.5 and 21.8% of 16:1*cis*7, respectively.

In summary, the ability of strain K1^T to inhibit the growth of *Vibrio anguillarum* and *A. salmonicida* seems to be a unique character among the carnobacteria. The closest known relative to strain K1^T, based on sequence data, is *C. alterfunditum*. The genus *Carnobacterium* is a phylogenetically coherent group (Wallbanks *et al.*, 1990) and the difference in 16S rDNA sequence between *C. alterfunditum* and strain K1^T is proposed to be sufficient to entitle a new species within the genus. The differences in fatty acid profiles and other phenotypic attributes support this conclusion. The name *Carnobacterium inhibens* is proposed for the new species.

Description of *Carnobacterium inhibens* sp. nov.

Carnobacterium inhibens (in.hi'bens. M.L. adj. *inhibens* from L. v. *inhibeo* to inhibit, referring to the growth-inhibitory activity that the bacterium shows).

Carnobacterium inhibens is a Gram-positive, motile (monotrichous), non-spore-forming rod which occurs singly or two, or as chains of four cells. Many standard laboratory media support growth but not MRS or TCBS. Colonies (TSA, 20 °C) are 1–2 mm in diameter, circular, entire, convex, and semi-translucent. The colour of the colonies is whitish at aerobic growth conditions and buff at anaerobic growth conditions. The temperature range that supports growth is 0–30 °C and the pH range that supports growth is 5.5–9.0. Does not produce catalase or oxidase but pseudo-catalase is produced on HBA. No gas is produced from glucose. Produces acid from sucrose, maltose, galactose, glucose, fructose, mannose, *N*-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, trehalose, lactose (weak), mannitol, ribose, inulin (weak), gentiobiose and methyl β -D-glucopyranoside, whereas it does not produce acid from cyclo-dextrin, glycerol, erythritol, xylose, adonitol, methylxyloside, sorbose, rhamnose, dulcitol, inositol, tagatose, D-arabitol, L-arabinose, melezitose, melibiose, pullulan, glycogen, raffinose, methyl D-mannoside, methyl D-glucoside, starch, xylitol, turanose, lyxose, fucose, gluconate and sorbitol. Does not produce acetoin or H₂S and is not able to reduce nitrate to nitrite. Hydrolyses hippurate. β -glucosidase activity is demonstrated whereas no activity of the following enzymes have been detected: urease, β -galactosidase, β -glucuronidase, α -galactosidase, arginine dihydrolase, alanyl-phenylalanyl-proline arylamidase, acid pyroglutamine arylamidase, *N*-acetyl- β -glucosaminidase. The most abundant cellular fatty acids are 16:0 (31.1%), 16:1 (24.2%) and 18:1cis9 (23.4%). Other fatty acids are 18:2cis9,12 or 18:0 (10.8%), 14:0 (5.4%) and 18:0 (3.5%). Atlantic salmon (*Salmo salar*) is a host for *C. inhibens*. *C. inhibens* is not known to be related to disease. *C. inhibens* has a unique 16S rDNA signature and the significance of the branching points in the unrooted phylogenetic tree (Fig. 1) is 73% (*C. funditum* S86170/*C. mobile* X54271), 98.1% (*C. alterfunditum* L08623/*C. inhibens* strain K1^T), 100% (*C. gallinarum* X54269/*C. piscicola* X54268) and 100% (*Vagococcus salmoninarum* X54272/*Vagococcus fluvialis* X54258). The *C. inhibens* type strain (K1^T) has been deposited at CCUG as 31728^T. The EMBL accession number for the 16S rDNA sequence is Z73313.

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