Chitinilyticum aquatile gen. nov., sp. nov., a chitinolytic bacterium isolated from a freshwater pond used for Pacific white shrimp culture

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Strain c14^T, originally isolated from surface water of a freshwater pond located in Pingtung (southern Taiwan) used for culture of Pacific white shrimp (*Litopenaeus vannamei*), was subjected to a polyphasic taxonomic approach. The strain exhibited strong chitinolytic activity and was able to grow under aerobic and anaerobic conditions by utilizing chitin exclusively as the carbon, nitrogen and energy source. Phylogenetic analysis of the 16S rRNA gene sequence revealed a clear affiliation of the proposed bacterium to the *Betaproteobacteria*, most closely related to *Chitinibacter tainanensis* S1^T, *Deefgea rivuli* WB 3.4-79^T and *Silvimonas terrae* KM-45^T, with 94.6, 93.6 and 92.9% 16S rRNA gene sequence similarity, respectively. The predominant fatty acids detected in cells of strain c14^T were C_{16:0}, C_{18:1ω7c} and summed feature 3 (C_{16:1ω7c} and/or C_{15:0} iso 2-OH). The G+C content of the genomic DNA was 69.5 (±1.0) mol%. Biochemical, physiological, chemotaxonomic and phylogenetic analyses showed that strain c14^T could not be assigned to any known genus of the *Betaproteobacteria*. Therefore, strain c14^T is classified within a novel genus and species, for which the name *Chitinilyticum aquatile* gen. nov., sp. nov. is proposed. The type strain of *Chitinilyticum aquatile* is c14^T (=LMG 23346^T =BCRC 17533^T).

Chitin is a common constituent of crustacean shells, cell walls of fungi, certain green algae and exoskeletons of insects and is the second most abundant polysaccharide in nature (after cellulose). It is an insoluble homopolymer composed of linear chains of β-1,4-linked *N*-acetyl-β-D-glucosamine (GlcNAc) residues, which are highly cross-linked by hydrogen bonds. Chitin and its partially deacetylated derivatives exhibit interesting properties and constitute a valuable raw material for biomedical, agricultural and cosmetic applications (Shigemasa & Minami, 1996). Annually, enormous amounts of chitin are synthesized in the biosphere, as much as 10¹⁰ to 10¹¹ metric tons (Gooday, 1990), but only a tiny minority remains in the

environment. The high turnover rate of the polysaccharide is attributed primarily to micro-organisms that degrade chitin, allowing carbon and nitrogen to return to the ecosystem (Gooday, 1990). It seems likely that shrimp culturing ponds could be a rich source of chitin-degrading bacteria. Since shrimp farming has contributed significantly to the economy of southern Taiwan, there are many types of shrimp ponds scattered around the Pingtung region. Some of them are very close to the coast, but some are far away from the sea, and that results in various water salinities in the ponds. Thus, the diverse culture types provide a variety of drastically different ecosystems in which micro-organisms can develop.

In our previous study, a strain of chitin-degrading bacteria, *Chitinimonas taiwanensis* cf^T, was isolated from a freshwater pond for shrimp (freshwater giant prawn, *Macrobrachium rosenbergii*) culture (Chang *et al.*, 2004). In this study, a water sample was collected from a freshwater pond for Pacific white shrimp (*Litopenaeus vannamei*) culture located in the Pingtung countryside in

Abbreviations: GlcNAc, *N*-acetyl-β-D-glucosamine; PHB, poly-β-hydroxybutyrate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain c14^T is DQ314581.

An HPLC profile of chitin degradation products of strain c14^T is available as supplementary material with the online version of this paper.

southern Taiwan. The temperature of the pond water was about 25 °C and the pH was approximately 7. The selected medium for isolation of chitin-degrading bacterium was enriched with 100 ml sample water with 0.5 % (w/v) autoclaved colloidal chitin. The initial culture was incubated at 25 °C with agitation at 125 r.p.m. Colloidal chitin was prepared from commercial chitin (Ohka Chemical) as described by Chang *et al.* (2004). After 5 days of incubation, the enrichment broth was diluted with sterile distilled water and spread onto chitin basal (CB) medium (Chang *et al.*, 2004). A bacterial strain that showed large clear zones around the colonies resulting from chitin degradation was isolated and designated c14^T. The colony morphology of strain c14^T was clearly different compared with that of *Chitinimonas taiwanensis* cf^T (Chang *et al.*, 2004). Consequently, the organism was subjected to a polyphasic taxonomic study that demonstrated its status as a member of a novel genus and species.

Bacterial cells were observed by phase-contrast microscopy (Leica DM 2000) in the lag, exponential and stationary phases of growth to ascertain their morphology. The motility of cells was examined by the hanging drop method. Flagellum staining was performed using the Spot Test flagella stain (BD Difco). Gram staining was performed using the Gram stain set S (BD Difco) and the Ryu non-staining KOH method (Powers, 1995). Poly- β -hydroxybutyrate (PHB) granule accumulation was observed by light microscopy after Sudan black staining. For scanning electron microscopy observations, specimens were fixed in 25 % glutaraldehyde in phosphate buffer (pH 7) for 20 min and then washed with phosphate buffer. Secondary fixation was done with 4 % osmium tetroxide in phosphate buffer for 40 min, again followed by a buffer rinse. After the secondary fix, dehydration was accomplished through a series of ethanol solutions (50, 70, 80, 90, 95 and 100 %); each change was held for 10 min. The specimens were then critical-point dried (Hitachi HCP-2), sputter-coated with gold (Hitachi E-1010) and examined with a digital scanning electron microscope (Hitachi S3500N).

The pH range for growth was determined by measuring optical densities (wavelength 595 nm) of the culture grown in tryptic soy broth (TSB; BD Difco). The pH of the medium was adjusted to pH 3–11 at intervals of 1.0 pH unit by using appropriate biological buffers: glycine/HCl, citrate/Na₂HPO₄, phosphate buffer and glycine/NaOH were used for pH values below 4, 4.0–8.0, 6.0–8.0 and 9.0–11.0, respectively. For the determination of the temperature range for growth, cells were incubated in CB broth at temperatures between 4 and 50 °C (4 °C and 10 to 50 °C at intervals of 5 °C) using an orbital water-bath shaker (125 r.p.m.). Tolerance of NaCl was determined by adjusting the salinity of CB medium to values between 0 and 3.0 % NaCl (w/v) (0, 0.25, 0.3, 0.5, 0.75, 1, 2 and 3 %). Anaerobic cultivation was performed on CB and R2A media in the Oxoid AnaeroGen system. Culture supernatant of strain c14^T was used to detect chitinolytic

production by HPLC and by the hydrolysis of fluorogenic substrate analogues [(GlcNAc)_n, where $n=1-6$; Sigma] as described previously (Chang *et al.*, 2004).

Light microscope investigations revealed that cells of strain c14^T were Gram-negative, rod-shaped, 0.3–0.4 μ m wide and 2.5–4.0 μ m long, and contained PHB granules. Cells were motile by means of single polar flagella. A scanning electron micrograph of strain c14^T is shown in Fig. 1.

Strain c14^T formed visible colonies (approx. 1.0–1.5 mm in diameter) on CB agar after 3 days of incubation at 35 °C. Colonies were cream-white in colour, circular and convex with entire edges. Surrounding the colonies, a colourless clear zone was observed that extended to more than double the colony diameter, indicating that chitinolysis was performed by strain c14^T during growth. With a medium containing only minerals and chitin, strain c14^T still exhibited a reasonable growth rate under both aerobic and anaerobic conditions, indicating that the organism could use chitin as a sole carbon, nitrogen and energy source for growth. In addition, vitamins and yeast extract were not needed as supplements for growth in mineral medium. Strain c14^T could also be grown on nutrient and tryptic soy media. Strain c14^T grew at temperatures ranging from 15 to 40 °C, pH values between 7 and 11 and NaCl concentrations between 0 and 0.75 %. Optimal growth conditions were 30–35 °C, pH 8 and 0.25 % NaCl. When the chitinolytic products were examined by HPLC and hydrolysis of fluorogenic substrates, the main product that remained in the c14^T culture medium was a disaccharide [chitodiose; (GlcNAc)₂], as shown in Supplementary Fig. S1, available in IJSEM Online. Clearly, strain c14^T could be distinguished from *Chitinimonas taiwanensis* cf^T, which produced chitotriose [(GlcNAc)₃] (Chang *et al.*, 2004), and *Chitinibacter tainanensis* S1^T, which produced GlcNAc monosaccharides (Chern *et al.*, 2004), as the major products of chitin cleavage.

Extraction of genomic DNA and PCR amplification and sequencing of the 16S rRNA gene were carried out as

Fig. 1. Electron micrograph of cells of strain c14^T. Bar, 1 μ m.

described previously (Chen *et al.*, 2001). Sequence reaction fragments were separated using a DNA sequencer (ABI PRISM 310 instrument; Applied Biosystems) and sequence assembly was done by using the Fragment Assembly System program from the Wisconsin Package 9.1 (Genetics Computer Group, 1995). The nearly complete 16S rRNA gene sequence of strain c14^T was compared with corresponding sequences taken from the GenBank and Ribosomal Database Project II databases. Multiple-sequence alignment including strain c14^T and its closest relatives was achieved using the BioEdit program (Hall, 1999) and MEGA version 3.1 (Kumar *et al.*, 2004). Phylogenetic reconstruction was inferred by using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms. An evolutionary distance matrix was generated for the neighbour-joining algorithm using the distance model of Jukes & Cantor (1969) and bootstrap analysis (1000 resamplings).

A nearly complete 16S rRNA gene sequence (1455 nt) of strain c14^T was obtained and revealed that strain c14^T belonged to the *Betaproteobacteria*. A phylogenetic tree inferred on the basis of evolutionary distances by using the neighbour-joining method (Fig. 2) indicated that strain c14^T formed a distinct lineage within the family *Neisseriaceae*. Sequence similarity calculations using pairwise alignment obtained from the EzTaxon database (Chun *et al.*, 2007) showed the greatest degree of similarity to *Chitinibacter tainanensis* S1^T (94.6% similarity) (Chern *et al.*, 2004), *Deefgea rivuli* WB 3.4-79^T (93.3%)

(Stackebrandt *et al.*, 2007), *Silvimonas terrae* KM-45^T (92.9%) (Yang *et al.*, 2005), *Formivibrio citricus* DSM 6150^T (92.4%) (Hippe *et al.*, 1999), *Iodobacter fluviatilis* ATCC 33051^T (92.2%) (Logan, 1989) and *Chitinimonas taiwanensis* cf^T (92.0%) (Chang *et al.*, 2004). The sequence similarity with other species belonging to the *Betaproteobacteria* was less than 92%. The overall topologies of the phylogenetic trees obtained with the neighbour-joining, maximum-likelihood and maximum-parsimony methods were similar.

Fluorometric DNA–DNA hybridization experiments were performed with photobiotin-labelled probes as described by Ezaki *et al.* (1989). Hybridization was conducted in 50% formamide at 50 °C. Each experiment was done in triplicate and the data are displayed as means of triplicate experiments. Strain c14^T showed relatively low DNA–DNA hybridization with its closest phylogenetic neighbour, *Chitinibacter tainanensis* BCRC 17254^T (21 ± 3%).

A DNA sample was prepared and degraded enzymically into nucleosides as described by Mesbah *et al.* (1989). The nucleoside mixture obtained was then separated by reversed-phase HPLC equipped with a Cosmosil 5C18 column (Waters). Non-methylated lambda phage DNA (Sigma) was used as the calibration reference. Each experiment was conducted in triplicate and the data are displayed as the mean of triplicate experiments. The G + C content of strain c14^T was 69.5 (± 1.0) mol%.

Cellular fatty acids were analysed in cells grown on TSB agar for 2 days. The cellular fatty acids were saponified and

Fig. 2. Neighbour-joining phylogenetic tree of strain c14^T and its relatives in the *Betaproteobacteria* based on 16S rRNA gene sequence comparisons. Bar, 1% sequence dissimilarity (1 substitution per 100 nucleotide positions). Bootstrap values (%) are indicated at branches from 1000 resamplings; only values >50% are shown. GenBank accession numbers are shown in parentheses. Filled circles indicate generic branches recovered by using maximum-parsimony.

methyated and then extracted according to the standard protocol of the Microbial Identification System (Sasser, 1990; MIDI Inc., 1999). The fatty acids analysed by GC (Hewlett Packard 6890) were identified by the Microbial Identification software package. The major cellular fatty acids of strain c14^T were C_{16:0}, C_{18:1ω7c} and summed feature 3 (C_{16:1ω7c} and/or C_{15:0} iso2-OH). The detailed fatty acid compositions of strain c14^T and its close phylogenetic neighbours are shown in Table 1. The fatty acid profile of strain c14^T was similar to those of *Chitinibacter tainanensis* S1^T, *S. terrae* KM-45^T, *D. rivuli* WB 3.4-79^T and *F. citricus* DSM 6150^T, which all contained predominantly C_{16:0}, C_{18:1ω7c} and summed feature 3, but differed in the proportions of fatty acids (Table 1). The respiratory quinone composition of strain c14^T (grown aerobically in CB broth at 30 °C) was analysed using previously described HPLC methods (Collins, 1985, 1994). Ubiquinones Q8 (extracted from *Escherichia coli* ATCC 11775^T) and Q10 (Sigma) were used as standards.

Table 1. Comparison of fatty acid profiles of strain c14^T and its phylogenetically closest relatives

Strains: 1, c14^T; 2, *Chitinibacter tainanensis* S1^T (data from Chern *et al.*, 2004); 3, *Silvimonas terrae* KM-45^T (Yang *et al.*, 2005); 4, *Deefgea rivuli* WB 3.4-79^T (Stackebrandt *et al.*, 2007); 5, *Formivibrio citricus* DSM 6150^T (Stackebrandt *et al.*, 2007). In order to obtain biomass for fatty acid extraction, strain c14^T, *Chitinibacter tainanensis* S1^T, *S. terrae* KM-45^T and *D. rivuli* WB 3.4-79^T were grown on TSB agar for 2 days at 30 °C; *F. citricus* DSM 6150^T was grown anaerobically on medium DSM 505. Values are percentages of total fatty acids; —, not detected/not reported.

Fatty acid	1	2	3	4	5
C _{12:0}	2.8	2.6	3.6	2.7	5.3
C _{12:0} 3-OH	2.6	3.9	—	2.5	—
C _{14:1ω5c}	0.8	—	—	—	—
C _{14:1}	—	—	—	—	1.2
C _{14:0}	2.5	0.5	6.4	2.0	1.6
C _{15:1ω8c}	—	0.3	—	—	—
C _{15:1ω6c}	—	0.3	—	1.5	—
C _{15:0}	—	0.8	—	2.9	—
C _{16:1ω5c}	0.7	0.6	—	—	—
C _{16:0}	23.0	16.7	33.5	20.7	11.9
C _{16:0} 3-OH	0.3	1.3	—	—	—
C _{17:0} cyclo	—	0.3	15.3	—	—
C _{17:1ω6c}	0.2	—	—	—	—
C _{17:0}	—	0.3	—	—	—
C _{18:1ω7c}	23.3	19.9	14.7	3.4	10.8
C _{18:1ω5c}	—	0.2	—	—	—
C _{18:0}	0.1	0.8	—	—	—
11-Methyl C _{18:1ω7c}	—	0.1	—	—	—
10-Methyl C _{19:0}	2.4	2.0	—	—	—
Summed feature 3*	41.3	48.6	21.8	60.1	57.7
Summed feature 4*	—	—	3.4	2.0	9.3

*Summed feature 3 comprises C_{16:1ω7c} and/or C_{15:0} iso 2-OH; summed feature 4 comprises C_{14:0} 3-OH and/or C_{16:1} iso I.

Ubiquinone 8 (Q8) was the only isoprenoid quinone present in strain c14^T.

Strain c14^T was characterized biochemically by using Biolog GN2 and API 20NE and API ZYM (bioMérieux) microtest systems according to the manufacturers' instructions. Catalase activity was determined by bubble production in a 10% (v/v) H₂O₂ solution. Oxidase activity was determined on filter paper moistened with a 1% (w/v) aqueous solution of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine. Strain c14^T was also examined for a broad range of phenotypic properties using conventional methods (MacFaddin, 2000). Susceptibility to antimicrobial agents was determined by a disc diffusion assay. The cell suspension was diluted to 0.5 McFarland standard in sterile saline after reaching the exponential growth phase and then spread onto CB medium and incubated at 35 °C. Antimicrobial discs (Difco) used in this experiment separately contained amikacin (30 µg), ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), novobiocin (30 µg), rifampicin (5 µg), penicillin G (10 U), streptomycin (10 µg) and tetracycline (30 µg). The effect of antibiotics on cell growth was assessed after 3 days of incubation at 35 °C and susceptibility was scored based on the distance from the edge of the clear zone to that of the disc (>3 mm, susceptible; 1–3 mm, moderately susceptible; <1 mm, resistant).

The differences in phenotypic and biochemical characteristics between strain c14^T and related phylogenetic neighbours are summarized in Table 2. Strain c14^T can be clearly distinguished from *Chitinibacter tainanensis* S1^T by the abilities to grow anaerobically, reduce nitrate, ferment glucose, hydrolyse gelatin and assimilate mannose. Differential phenotypic characteristics between strain c14^T and *S. terrae* KM-45^T include that strain c14^T can hydrolyse gelatin and lacks activities of β-galactosidase and β-glucosidase. Strain c14^T can be clearly distinguished from *D. rivuli* WB 3.4-79^T by its abilities to ferment glucose, hydrolyse chitin, grow at 40 °C and hydrolyse gelatin. While strain c14^T is facultatively anaerobic, *F. citricus* DSM 6150^T was described as strictly anaerobic (Tanaka *et al.*, 1991).

It is now generally accepted that bacteria with a G+C content difference of more than 10 mol% should not be classified within the same genus (Wayne *et al.*, 1987). Also, 16S rRNA sequence similarities between two bacteria of less than 95% are an indication of affiliation to different genera (Ludwig *et al.*, 1998). Strain c14^T most likely represents a species of a new genus, since the 16S rRNA gene sequence similarity to its closest relatives with validly published names, *Chitinibacter tainanensis* S1^T, *S. terrae* KM-45^T and *D. rivuli* WB 3.4-79^T, is 94.4, 92.7 and 92.6%, respectively. The G+C content of c14^T (69.5 mol%) is also significantly higher than those of *Chitinibacter tainanensis* S1^T (56 mol%), *S. terrae* KM-45^T (58 mol%) and *D. rivuli* WB 3.4-79^T (48.5 mol%). Moreover, strain c14^T can be

Table 2. Difference in phenotypic and biochemical characteristics between strain c14^T and its phylogenetically closest relatives

Strains: 1, c14^T; 2, *Chitinibacter tainanensis* S1^T (data from Chern *et al.*, 2004); 3, *S. terrae* KM-45^T (Yang *et al.*, 2005); 4, *D. rivuli* WB 3.4-79^T (Stackebrandt *et al.*, 2007); 5, *F. citricus* DSM 6150^T (Tanaka *et al.*, 1991; Hippe *et al.*, 1999; data for chitin hydrolysis taken from Chern *et al.*, 2004). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5
Source	Freshwater	Soil	Soil	Hard-water creek	Anoxic freshwater mud
Morphology	Rods, straight to slightly curved	Rods, straight to slightly curved	Rods, single or in pairs	Rods, straight to slightly curved	Curved rods, single or in pairs
Relation to O ₂	Facultatively anaerobic	Strictly aerobic	Facultatively anaerobic	Facultatively anaerobic	Strictly anaerobic
Catalase	+	+	+	+	–
Chitin hydrolysis	+	+	+	–	–
Growth at 40 °C	+	+	+	–	–
API 20NE results					
Nitrate reduction	+	–	+	+	ND
Fermentation of glucose	+	–	+	–	ND
Gelatin hydrolysis	+	–	–	–	ND
β-Glucosidase	–	–	+	–	ND
β-Galactosidase	–	–	+	–	ND
Maltose assimilation	–	–	+	–	ND
Mannose assimilation	+	–	+	–	ND
N-Acetylglucosamine assimilation	+	+	+	+	ND
Gluconate assimilation	+	+	+	+	ND
DNA G + C content (mol%)	69.5	56	58	48.5	61

readily distinguished from its nearest phylogenetic neighbours, *Chitinibacter tainanensis* S1^T, *S. terrae* KM-45^T, *D. rivuli* WB 3.4-79^T and *F. citricus* DSM 6150^T, by fatty acid compositions and phenotypic and biochemical characteristics. Therefore, based on phenotypic and phylogenetic criteria, we are of the opinion that strain c14^T isolated from surface water of a freshwater pond for shrimp culture should be assigned to a novel species of a new genus, for which the name *Chitinilyticum aquatile* gen. nov., sp. nov. is proposed.

Description of *Chitinilyticum* gen. nov.

Chitinilyticum (Chi.ti.ni.ly'ti.cum. N.L. n. *chitinum* chitin; N.L. adj. *lyticus* -a -um dissolving, used as subst. dissolver; N.L. neut. n. *Chitinilyticum* chitin-dissolver).

Cells are Gram-negative rods, motile by means of single polar flagella. PHB granules are stored as reserve material. Endospores are not formed. Colonies on CB medium display a large clear zone around them, which is derived from degradation of chitin. No diffusible pigments are produced. Grow well by using chitin as the sole carbon, nitrogen and energy source under both aerobic and anaerobic conditions. Positive for catalase and oxidase. Major fatty acid components are C_{16:0}, C_{18:1ω7c} and summed feature 3 (C_{16:1ω7c} and/or C_{15:0} iso 2-OH). Major isoprenoid quinone is Q8. The G + C content of the DNA of the type strain of the type species is 69.5 mol%. Based on 16S rRNA gene sequence analysis, *Chitinilyticum* belongs to the class *Betaproteobacteria*, family *Neisseriaceae*,

showing a distant relatedness to *Chitinibacter tainanensis*, *Silvimonas terrae*, *Deefgea rivuli* and *Formivibrio citricus*. The type species is *Chitinilyticum aquatile*.

Description of *Chitinilyticum aquatile* sp. nov.

Chitinilyticum aquatile (L. neut. adj. *aquatile* living in water).

Displays the following properties in addition to those given in the genus description. Cells are 2.5–4.0 µm long and 0.3–0.4 µm in diameter, occurring singly. Growth occurs at 15–40 °C, pH 7–11 and 0–0.75 % NaCl. Positive for glucose fermentation. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H₂S production (triple-sugar iron agar and sulfide indole motility medium), acetoin production (Voges–Proskauer test) and fermentation of mannitol, sucrose, arabinose and rhamnose. API 20NE tests show positive reactions for nitrate reduction, glucose fermentation, gelatin hydrolysis and assimilation of glucose, mannose, N-acetyl-D-glucosamine and gluconate and negative reactions for indole production, arginine dihydrolase, urease, aesculin hydrolysis, β-galactosidase and assimilation of arabinose, mannitol, maltose, caprate, adipate, malate, citrate and phenylacetate. In API ZYM tests, shows positive results for alkaline phosphatase, C4 esterase, C8 lipase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase and negative reactions for α-glucosidase,

β -glucuronidase, α -galactosidase, β -galactosidase, β -glucosidase, C14 lipase, valine arylamidase, cystine arylamidase, α -mannosidase, α -fucosidase, trypsin and α -chymotrypsin. The following carbon sources are oxidized (Biolog GN2): dextrin, glucose, D-fructose, sucrose, cellobiose, mannose, trehalose, turanose, gluconate, D-glucuronic acid, acetic acid, α -ketobutyric acid, α -ketovaleric acid, DL-lactate, N-acetyl-D-glucosamine, α -hydroxybutyric acid, glucose 6-phosphate and L-alanine. The following substrates are not utilized (Biolog GN2): α -cyclodextrin, glycogen, Tween 40, Tween 80, arabinose, maltose, lactulose, psicose, D-melibiose, itaconic acid, L-fucose, inosine, α -D-lactose, D-galactose, D-raffinose, gentiobiose, L-rhamnose, mannitol, xylitol, glycerol, arabitol, *i*-erythritol, D-galacturonic acid, D-sorbitol, *myo*-inositol, adonitol, 2,3-butanediol, N-acetyl-D-galactosamine, DL- α -glycerol phosphate, glucose 1-phosphate, methyl β -D-glucoside, 2-aminoethanol, monomethyl succinate, citrate, formic acid, D-galactonic acid lactone, methyl pyruvate, malonic acid, α -ketoglutaric acid, propionic acid, urocanic acid, D-saccharic acid, succinic acid, bromosuccinic acid, succinamic acid, *p*-hydroxyphenylacetic acid, *cis*-aconitic acid, quinic acid, sebacic acid, γ -aminobutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, D-glucosaminic acid, phenyl ethylamine, L-glutamic acid, L-histidine, L-aspartic acid, L-leucine, L-threonine, L-phenylalanine, L-asparagine, L-serine, D-alanine, D-serine, L-proline, hydroxy-L-proline, glycyl L-glutamic acid, L-ornithine, DL-carnitine, L-pyroglyutamic acid, glycyl L-aspartic acid, L-alanyl glycine, putrescine, alaninamide, glucuronamide, thymidine and uridine. Sensitive to amikacin (30 μ g), ampicillin (10 μ g), chloramphenicol (30 μ g), kanamycin (30 μ g), nalidixic acid (30 μ g), novobiocin (30 μ g), rifampicin (5 μ g) and tetracycline (30 μ g). Resistant to erythromycin (15 μ g), gentamicin (10 μ g), penicillin G (10 U) and streptomycin (10 μ g).

The type strain, c14^T (=BCRC 17533^T =LMG 23346^T), was isolated from surface water of an aquaculture pond containing Pacific white shrimp (*Litopenaeus vannamei*) at Pingtung in southern Taiwan.

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