Assignment of *Eubacterium* sp. VPI 12708 and related strains with high bile acid 7 α -dehydroxylating activity to *Clostridium scindens* and proposal of *Clostridium hylemonae* sp. nov., isolated from human faeces

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Unknown *Eubacterium*-like organisms VPI 12708 and five strains (Y-1113, I-10, M-18, TH-82 and 36S) had high bile acid 7 α -dehydroxylating activity; the unknown *Clostridium*-like organisms TN-271^T and TN-272 also had the same activity. Analysis of their 16S rDNA sequences demonstrated that all strains belong to cluster XIVa of the genus *Clostridium* (Collins *et al.*, 1994). Strain VPI 12708 and five other strains (Y-1113, I-10, M-18, TH-82 and 36S) formed a single cluster and strains TN-271^T and TN-272 formed another single cluster. *Clostridium scindens* JCM 6567^T was the most closely related species for two clusters in the phylogenetic tree. Values for DNA–DNA similarities among *C. scindens* JCM 6567^T, strain VPI 12708 and the other five strains were greater than 70%, showing that these micro-organisms were a single species. Therefore, we identified strain VPI 12708 and the five other strains as *C. scindens*. In addition, DNA–DNA similarities among *C. scindens* JCM 6567^T, strain TN-271^T and strain N-272 revealed that strains TN-271^T and TN-272 were distinct from *C. scindens* JCM 6567^T. On the basis of phylogenetic analysis and DNA–DNA similarity data, it was concluded that strains TN-271^T and TN-272 are members of a new species of the genus *Clostridium*, for which the name *Clostridium hylemonae* is proposed. The type strain is strain TN-271^T (= JCM 10539^T).

Keywords: bile acid, 7 α -dehydroxylation, *Clostridium scindens*, *Eubacterium* sp. VPI 12708, *Clostridium hylemonae*

INTRODUCTION

Members of the genera *Clostridium* and *Eubacterium* are the predominant intestinal species exhibiting bile acid 7 α -dehydroxylating activity (Archer *et al.*, 1982; Ferrari *et al.*, 1977; Hayakawa & Hattori, 1970; Hirano *et al.*, 1981; Stellwag & Hylemon,

1979; Takamine & Imamura, 1995). The presence of this activity in the intestinal microflora results in 7 α -dehydroxylation of cholic acid and chenodeoxycholic acid, yielding deoxycholic acid and lithocholic acid, respectively. Secondary bile acids have long been implicated in colorectal cancer as co-carcinogens (Batta *et al.*, 1998; Cheah & Bernstein, 1990; Hill, 1975; Mastromarino *et al.*, 1976; Reddy, 1981; Reddy *et al.*, 1977), indicating that bile acid 7 α -dehydroxylation is an important physiological reaction in the intestinal ecosystem.

Strain VPI 12708, an isolate from human faeces, was reported to exhibit bile acid 7 α -dehydroxylating ac-

The GenBank/EMBL/DBJ accession numbers for the 16S rDNA sequences reported in this paper are as follows: AB020883 (*Clostridium scindens* JCM 6567^T), AB020727 (*C. scindens* VPI 12708), AB020728 (*C. scindens* Y-1113), AB020729 (*C. scindens* M-18), AB020730 (*C. scindens* I-10), AB020731 (*C. scindens* TH82), AB020806 (*C. scindens* 36S), AB023972 (*Clostridium hylemonae* TN-271^T) and AB023973 (*C. hylemonae* TN-272).

tivity and was identified as a member of the genus *Eubacterium* (White *et al.*, 1980). After the characterization, many studies (Baron *et al.*, 1991; Coleman *et al.*, 1987; Doerner *et al.*, 1997; Franklund *et al.*, 1993; Mallonee & Hylemon, 1996; Mallonee *et al.*, 1990, 1992) on strain VPI 12708, especially on the *bai* (bile acid inducible) operon, have been reported. However, detailed studies concerning taxonomic research on strain VPI 12708 have not appeared. Takamine & Imamura (1995) isolated and characterized some strains of bile acid 7 α -dehydroxylating bacteria and concluded that a few strains were members of the genus *Eubacterium*. Doerner *et al.* (1997) assessed bile acid 7 α -dehydroxylating bacteria for the presence of a *bai*-like gene by Southern hybridization. They found three types of bile acid 7 α -dehydroxylating bacteria: one type possessed all of the *bai* operon; one possessed none of the operon; and another possessed only some parts of the operon.

We consider that the taxonomic positions of strain VPI 12708 and other isolates from faeces of healthy humans should be clarified to determine which species are responsible for the bile acid 7 α -dehydroxylation reaction. The purpose of this study was to examine the taxonomic positions of strain VPI 12708 and other bile acid 7 α -dehydroxylating bacteria by means of 16S rDNA sequencing and DNA–DNA similarity experiments.

METHODS

Bacterial strains and cultivation. Several bacterial strains with known bile acid 7 α -dehydroxylating activity were used in this study (Table 1). Strain VPI 12708 was originally isolated by R. Hammann from the faeces of a colon-cancer patient. The media and techniques used were as described previously (White *et al.*, 1980). The other strains (Y-1113, I-10, M-18, TH-82, 36S, TN-271^T and TN-272) were isolated by F. Takamine from the faeces of healthy humans, using previously described procedures (Doerner *et al.*, 1997).

All of the bacterial strains were cultured on Eggerth–Gagnon (EG; Eiken) blood agar plates with 5% horse blood, GAM agar plates (Nissui) and egg-yolk agar plates for 2 d at 37 °C in anaerobic jar boxes (Hirayama) filled with 100% CO₂. Egg-yolk agar plates contained the following: 20 g peptone, 2.5 g Na₂PO₄, 0.5 g K₂HPO₄, 1 g NaCl, 0.05 g MgSO₄, 1 g glucose and 12.5 g agar in 500 ml distilled water (pH 7.6) and 50 ml 50% egg-yolk solution.

Physiological and biochemical tests. The basal medium for physiological and biochemical tests was PYF medium. The PYF medium was composed of 1000 ml containing the following: 10 g trypticase (BBL; Becton Dickinson), 10 g yeast extract (Difco), 40 ml Fildes' solution, 40 ml salts solution and 0.5 g L-cysteine.HCl.H₂O; the pH was adjusted to 7.6. The salts solution contained 0.2 g CaCl₂, 0.2 g MgSO₄, 1 g K₂HPO₄, 1 g KH₂PO₄, 10 g NaHCO₃, 2 g NaCl and 1000 ml distilled water. The Fildes' solution contained 150 ml physiological saline, 6 ml concentrated HCl, 50 ml horse blood and 1 g pepsin (1:10000; Difco); the pH was adjusted to 7.6 after digestion.

H₂S production, indole production, nitrate reduction, motility, aesculin hydrolysis, starch hydrolysis and gelatin

liquefaction were detected by using the methods of Holdman *et al.* (1977).

Acid production from 30 sugars was determined by using PYF medium containing 0.5% (w/v) sugar, excluding amygdalin and aesculin (0.25%). The pH was measured directly in the culture tubes by using a combination electrode (an automatic, multipoint pH-measuring system; Lifetech). The final pH was determined after incubation for 7 d.

The metabolic end products were analysed by means of GLC (GC-14A; Shimadzu) using a 2.1 m glass column (i.d. 2.8 mm, FAL-M 25%, Chrommosorb W, AW-DMCS H₂PO₄, 80/100 mesh). The short fatty acids were analysed by means of acidified ether-extraction.

The enzymic activity tests were performed using API ZYM (API bioMérieux) according to the instructions of the manufacturer.

16S rDNA analysis. Fragments (approx. 1500 bases) of the 16S rDNA genes of strains used in this study were amplified by PCR with primers 5'-dAGAGTTTGATCCTGGCT-CAG-3' (designated primer 27F) and 5'-dGGTTACCT-TGTTACGACTT-3' (designated primer 1492R), using PCR Thermal Cycler MP (Takara Shuzo). The PCR products were purified using a GENE Mate PCR pure SPIN purification kit (Intermountain Scientific Corporation) and were sequenced using an AutoCycle sequencing kit (Pharmacia Biotech) and an ALF express DNA sequencer (Pharmacia Biotech). The new sequences were compared with the sequences of reference organisms from the GenBank database. Phylogenetic analysis was performed with the software CLUSTAL W (Thompson *et al.*, 1994) and a phylogenetic tree was constructed according to the neighbour-joining method (Saitou & Nei, 1987). The topology of the tree was evaluated by bootstrap analysis using the CLUSTAL W program (Thompson *et al.*, 1994).

Nucleotide sequence accession numbers. The 16S rDNA sequences of strain VPI 12708 and five strains have been deposited in the DDBJ database. The following 16S rRNA/rDNA sequences were used for the phylogenetic analysis: *Clostridium aerotolerans* DSM 5434^T (X76163), *Clostridium aminophilum* VPI 14602 (L04165), *Clostridium aminovalericum* DSM 1283^T (X73436), *Clostridium celerecrescens* DSM 5628^T (X71848), *Clostridium clostridiiforme* ATCC 25537^T (M59089), *Clostridium coccooides* DSM 2088 (M59090), *Clostridium nexile* DSM 1787^T (X73443), *Clostridium oroticum* ATCC 13619^T (M59109), *Clostridium polysaccharolyticum* DSM 1801^T (X71858), *Clostridium populeti* ATCC 35295^T (X71853), *Clostridium sphenoides* DSM 632^T (X73449), *Clostridium symbiosum* ATCC 14940^T (M59112), *Clostridium xylanolyticum* ATCC 49623^T (X71855), *Coprococcus eutactus* ATCC 27759^T (D14148), *Eubacterium cellulosolvens* ATCC 43171^T (L34613), *Eubacterium formicigenerans* ATCC 27755^T (L34619), *Eubacterium limosum* KIST 612 (U67159), *Roseburia cecicola* ATCC 33874^T (L14676), *Ruminococcus hansenii* ATCC 27752^T (D14155), *Ruminococcus productus* ATCC 27340^T (L76595) and *Ruminococcus torques* ATCC 27756^T (L76604).

DNA base composition. DNAs were extracted from the cells harvested from EGF broth after growth for 12 h at 37 °C and purified by the methods of Saito & Miura (1963). DNA base compositions were determined using HPLC (Tamaoka & Komagata, 1984) after enzyme digestion of DNA with deoxyribonucleosides. An equimolar mixture of four deoxy-

Table 1. Strains used in this study

Strain*	Source	Reference	Presence of <i>bai</i> genes†					
			<i>baiB</i>	<i>baiE</i>	<i>baiA2</i>	<i>baiG</i>	<i>baiH</i>	<i>baiI</i>
<i>Clostridium scindens</i> JCM 6567 ^T	Faeces of a healthy human adult	Morris <i>et al.</i> (1985)	+	+	+	+	+	+
VPI 12708 (= JCM 10418)	Faeces of a colon-cancer patient	White <i>et al.</i> (1980)	+	+	+	+	+	+
Y-1113 (= JCM 10419)	Faeces of a healthy human adult	Takamine & Imamura (1995)	+	+	+	+	+	+
M-18 (= JCM 10420)	Faeces of a healthy human adult	Takamine & Imamura (1995)	NT	+	NT	NT	NT	NT
I-10 (= JCM 10421)	Faeces of a healthy human adult	Doerner <i>et al.</i> (1997)	NT	+	NT	NT	NT	NT
TH82 (= JCM 10422)	Faeces of a healthy human adult	Doerner <i>et al.</i> (1997)	+	+	+	+	+	+
36S (= JCM 10423)	Faeces of a healthy human adult	Takamine & Imamura (1995)	+	+	+	+	+	+
TN-271 ^T (= JCM 10539 ^T)	Faeces of a healthy human adult	Doerner <i>et al.</i> (1997)	—	—	+	+	+	+
TN-272 (= JCM 10540)	Faeces of a healthy human adult	None	—	—	+	+	+	+

*JCM, Japan Collection of Microorganisms; VPI, Virginia Polytechnic Institute; superscript T indicates that the strain is the type strain.

†According to Doerner *et al.* (1997); NT, not tested.

ribonucleotides in the GC kit (Yamasa Shoyu) was used as the quantitative standard.

DNA–DNA similarity. Levels of DNA–DNA similarity were determined by the method of Ezaki *et al.* (1989), using photobiotin and microplates.

RESULTS

Biological characterization

The spores of *Clostridium scindens* JCM 6567^T were very difficult to demonstrate microscopically but were observed in strains from egg-yolk agar plates. Spore formation was not observed in strain VPI 12708 or in the other five strains (Y-1113, I-10, M-18, TH-82 and 36S), even from egg-yolk agar plates. The colony- and cell morphologies of strain VPI 12708 and the five strains were very similar to those of *C. scindens* JCM 6567^T.

In strains TN-271^T and TN-272, spore formation was observed on EG agar plates. The colony- and cell morphologies of strains TN-271^T and TN-272 were very similar to those of *C. scindens* JCM 6567^T (Fig. 1).

Physiological and biochemical properties

The characteristics of the *C. scindens* and *Clostridium hylemonae* strains are summarized in Table 2. Strain VPI 12708 and the other five strains produced acid from fructose, glucose and ribose but not from adonitol, amygdalin, dulcitol, erythritol, aesculin, glycerol, glycogen, inositol, inulin, mannitol, melezitose, melibiose, rhamnose, salicin, sorbitol, sorbose,

Fig. 1. Light micrograph of strain TN-271^T showing terminal spores. Cells were cultured on GAM agar. Bar, 10 μ m.

starch or trehalose. With regard to other sugars, each strain showed different sugar-fermentation patterns. With regard to biochemical properties, strain VPI 12708 and the other five strains showed gas formation, did not produce indole, did not reduce nitrate and did not liquefy gelatin. Only strain I-10 hydrolysed aesculin and starch. Strains Y-1113, M18 and 36S produced H₂S.

Strains TN-271^T and TN-272 produced acid from galactose, glucose, raffinose and sucrose, but not from adonitol, amygdalin, cellobiose, dulcitol, erythritol, aesculin, glycerol, glycogen, inositol, inulin, lactose, mannitol, mannose, melezitose, melibiose, rhamnose, salicin, sorbitol, sorbose, starch or trehalose. For arabinose, fructose, maltose, ribose and xylose, strains

Table 2. Characteristics of *Clostridium scindens* and *Clostridium hylemonae*

All strains of *C. scindens* and *C. hylemonae* produced acid from glucose.

Characteristic	<i>C. scindens</i>							<i>C. hylemonae</i>	
	JCM 6567 ^T	I-10	Y-1113	M18	VPI 12708	36S	TH82	TN-271 ^T	TN-272
Spore formation	+	—	—	—	—	—	—	+	+
H ₂ S production	—	—	+	+	—	+	—	+	+
Aesculin hydrolysis	—	+	—	—	—	—	—	—	—
Starch hydrolysis	—	+	—	—	—	—	—	—	—
Acid production from:*									
Arabinose	—	—	+	+	+	+	+	+	—
Xylose	—	—	+	+	+	+	+	+	—
Ribose	+	+	+	+	+	+	+	+	—
Mannose	+	+	—	—	—	—	+	—	—
Fructose	+	+	+	+	+	+	+	+	—
Galactose	+	+	+	—	+	+	+	+	+
Sucrose	—	—	+	+	—	+	+	+	+
Maltose	—	—	—	—	—	—	+	+	—
Cellobiose	—	+	—	—	—	—	+	—	—
Lactose	+	+	—	—	+	—	+	—	—
Raffinose	—	—	—	—	—	—	+	+	+

* The fermentation tests were determined as positive (+) when the pH of the medium was acidified more than 1.0 pH unit below that of the control without carbohydrates.

Table 3. Enzymic activities of *C. hylemonae* and *C. scindens*

Enzymic tests were performed with an API 20S system according to the instructions of the manufacturer. The quantities of hydrolysed substrate were as follows: +, >20 nmol; (+), 1–20 nmol; —, <10 nmol. 1, Alkaline phosphatase; 2, naphthol-AS-BI-phosphohydrolase; 3, α -galactosidase; 4, β -galactosidase; 5, α -glucosidase. All of the strains shown gave the same results for the following enzyme activities (each result is given in parenthesis): esterase (+), esterase lipase (+), lipase (—), leucine arylamidase (—), valine arylamidase (—), cystine arylamidase (—), trypsin (—), chymotrypsin (—), phosphatase (+), β -glucuronidase (—), β -glucosidase (—), *N*-acetyl- β -glucosamidase (—), α -mannosidase (—) and α -fucosidase (—).

Strain	1	2	3	4	5
<i>C. scindens</i> JCM 6567 ^T	—	+	—	+	—
<i>C. scindens</i> VPI 12708	—	+	—	+	—
<i>C. scindens</i> I-10	—	+	—	+	—
<i>C. scindens</i> Y-1113	(+)	+	—	—	—
<i>C. scindens</i> M18	—	—	—	—	—
<i>C. scindens</i> 36S	—	—	—	—	—
<i>C. scindens</i> TH82	—	—	—	—	—
<i>C. hylemonae</i> TN-271 ^T	(+)	+	(+)	—	+
<i>C. hylemonae</i> TN-272	(+)	+	(+)	—	+

TN-271^T and TN-272 showed different sugar-fermentation patterns. With regard to biochemical properties, strains TN-271^T and TN-272 did not produce indole,

did not reduce nitrate, did not liquefy gelatin and did not hydrolyse aesculin or starch. Strains TN-271^T and TN-272 showed gas formation and H₂S production.

API ZYM analysis

The enzymic activities of the *C. scindens* and *C. hylemonae* strains are shown in Table 3. Strains VPI 12708 and I-10 had the same enzyme-activity pattern as *C. scindens* JCM 6567^T, but strains Y-1113, M18, 36S and TH82 had different patterns of enzyme activity relative to *C. scindens* JCM 6567^T. Strains TN-271^T and TN-272 gave positive reactions on α -galactosidase and α -glucosidase, while all strains of *C. scindens* gave negative reactions.

16S rDNA sequence analysis

The 16S rDNA sequence of each strain was determined for approximately 1500 bases. It was clear that *C. scindens* JCM 6567^T was positioned in cluster XIVa of the genus *Clostridium* (Collins *et al.*, 1994). Strains VPI 12708, Y-1113, I-10, M-18, TH-82 and 36S formed a single cluster and *C. scindens* JCM 6567^T was the most closely related species in the phylogenetic tree. The level of sequence similarity between *C. scindens* JCM 6567^T and strain VPI 12708 was 99.0%.

Strains TN-271^T and TN-272 formed a single cluster and *C. scindens* JCM 6567^T was the most closely related species in the phylogenetic tree, but the level of sequence similarity between *C. scindens* JCM 6567^T and strain TN-271^T was only 91.3%. The phylogenetic

Fig. 2. Phylogenetic relationships within *Clostridium* cluster XIVa and closely related species. The tree was created by using the neighbour-joining method and K_{nuc} values. The numbers on the tree indicate bootstrap values greater than 50%. Bar, 0.02 K_{nuc} .

tree of clostridial cluster XIVa created with the representative strains is shown in Fig. 2.

DNA base composition and DNA–DNA similarity

DNA base compositions and levels of DNA–DNA similarity are shown in Table 4. *C. scindens* JCM 6567^T, strains VPI 12708 and the other five strains (Y-1113, I-10, M-18, TH-82 and 36S) had G+C contents in the range 46.0–47.2 mol%. The reassociation of

DNA among *C. scindens* JCM 6567^T, strains VPI 12708, Y-1113, I-10, M-18, TH-82 and 36S was greater than 70%. These findings clearly revealed that *C. scindens* JCM 6567^T, VPI 2708 and the other five strains were related genetically and that they formed a single species, as *C. scindens*.

Strains TN-271^T and TN-272 had the same G+C content (48.6 mol%). The reassociation of DNA between strains TN-271^T and TN-272 was greater than

Table 4. DNA base composition and levels of DNA–DNA similarity among *C. scindens* and *C. hylemonae* strains

Strain	G + C content (mol %)	DNA–DNA reassociation (%) with:								
		JCM 6567 ^T	VPI 12708	36S	Y-1113	TH82	I-10	M18	TN-271 ^T	TN-272
<i>C. scindens</i>										
JCM 6567 ^T	46.5	100	85	78	88	96	100	84	8	5
VPI 12708	47.1	95	100	97	101	88	94	112	11	7
36S	46.9	84	96	100	98	75	82	109	8	5
Y-1113	47.1	89	100	100	100	79	89	108	10	6
TH82	46.0	95	83	75	79	100	102	97	7	4
I-10	47.2	88	77	70	72	91	100	88	5	3
M18	47.1	78	91	91	93	70	70	100	5	3
<i>C. hylemonae</i>										
TN-271 ^T	48.6	15	19	14	17	13	14	11	100	98
TN-272	48.6	20	22	16	19	15	18	12	103	100

98 %, while that between strain TN-271^T and strains of *C. scindens* was less than 19 %. It clearly revealed that strains TN-271^T and TN-272 were distinct from *C. scindens*.

DISCUSSION

Many studies (Archer *et al.*, 1982; Ferrari *et al.*, 1977; Hayakawa & Hattori, 1970; Hirano *et al.*, 1981; Stellwag & Hylemon, 1979; Takamine & Imamura, 1995; White *et al.*, 1980) on faecal bacteria having 7 α -dehydroxylating activity have been reported. In these reports, the multistep bile acid 7 α -dehydroxylation pathway was studied in detail only for strain VPI 12708 (Baron *et al.*, 1991; Coleman *et al.*, 1987; Doerner *et al.*, 1997; Franklund *et al.*, 1993; Mallonee & Hylemon, 1996; Mallonee *et al.*, 1990, 1992). However, strain VPI 12708 was identified as a member of the genus *Eubacterium*; the detailed taxonomic position has not been reported. As in the case of strain VPI 12708, other bile acid 7 α -dehydroxylating bacteria (strains Y-1113, I-10, M-18, TH-82, 36S, TN-271^T and TN-272) that were isolated and characterized (Doerner *et al.*, 1997; Takamine & Imamura, 1995) have been identified only as members of the genera *Eubacterium* and *Clostridium* and have not been identified at species level. The precise taxonomic positions of bile acid 7 α -dehydroxylating bacteria remain to be identified in the course of further studies.

In this study, we handled two groups on the basis of the hybridization results with each part of the *bai* operon. One is a group of strains (VPI 12708, Y-1113, TH82 and 36S) that possess all parts of the *bai* operon, while the other is a group of strains (TN-271^T and TN-272) that possess four out of the six parts of the *bai* operon.

Strain VPI 12708 and the other five strains (Y-1113, I-10, M-18, TH-82 and 36S) had different patterns of sugar fermentation and enzymic activity. However, the

16S rDNA sequence analysis showed that they were all closely related to *C. scindens* JCM 6567^T on the phylogenetic tree presented in Fig. 2. The similarity level of the 16S rDNA sequences between strain VPI 12708 and *C. scindens* JCM 6567^T (99.0 %) supported this phylogenetic relationship. The phylogenetic tree further demonstrated that the *C. scindens* type strain belongs to cluster XIVA of the genus *Clostridium*, although its 16S rDNA sequence has not been deposited in the DDBJ database. As shown in Table 4, the DNA–DNA hybridization results demonstrated that strain VPI 12708 and the five strains had inter-group DNA similarity values of above 70.0 %, indicating that this group was composed of a single species that could be assigned to *C. scindens*.

Analysis of the 16S rDNA also showed that strains TN-271^T and TN-272 were closely related to *C. scindens* JCM 6567^T. However, as shown in Table 4, the levels of reassociation among strains TN-271^T, TN-272 and *C. scindens* JCM 6567^T demonstrated that TN-271^T and TN-272 were clearly distinct from *C. scindens* JCM 6567^T and belong to a new species of the genus *Clostridium*, for which we propose the name *Clostridium hylemonae*. The enzymic activities of α -galactosidase and α -glucosidase represent a useful method for phenotypically distinguishing between *C. scindens* and *C. hylemonae*, as shown in Table 3.

It is concluded that the taxonomic study of 16S rDNA was consistent with the *bai* gene hybridization pattern. Strains that possess all parts of the *bai* gene belong to *C. scindens* and strains that possess only some parts of this gene belong to a new species, i.e. *C. hylemonae*.

Thus, *C. scindens* and *C. hylemonae* are noted as bile acid 7 α -dehydroxylating bacteria of the human intestinal microflora. Furthermore, by the quantitative detection of *C. scindens* and *C. hylemonae* in the faeces of healthy humans and of colon-cancer patients, it may be possible to establish if bile acid 7 α -dehydroxylation

of *C. scindens* and *C. hylemonae* is correlated with an increased risk of colon cancer.

Description of *Clostridium hylemonae* sp. nov.

Clostridium hylemonae (hai.le.mon'ae. N.L. gen. n. *hylemonae* of Hylemon, after the American microbiologist Phillip B. Hylemon, for his contributions to research on bile acid).

The description of the characteristics given below is based on the results of studies with the two strains TN-271^T and TN-272. Cells are Gram-positive, spore-forming and non-motile. The straight or slightly curved, rod-shaped cells are 1.0–5.3 × 0.2–0.5 µm in size and occur singly or in pairs. Colonies are 0.5–1.0 mm in diameter, disc-shaped and greyish in colour. The optimum temperature for growth is 37 °C and the optimum pH is around 7.6. Obligately anaerobic. Two strains produce acid from galactose, glucose, raffinose and sucrose and have variable reactions with arabinose, fructose, maltose, ribose and xylose. None of the strains produce acid from adonitol, amygdalin, cellobiose, dulcitol, erythritol, aesculin, glycerol, glycogen, inositol, inulin, lactose, mannitol, mannose, melezitose, melibiose, rhamnose, salicin, sorbitol, sorbose, starch or trehalose. H₂S and gas are produced. Indole is not produced. Nitrate is not reduced. Aesculin and starch are not hydrolysed. Gelatin is not liquefied. Moderate amounts of acetic acid (as the endproduct) are produced in peptone/yeast extract medium supplemented with glucose; minor amounts of propionic acid, iso-butyric acid, butyric acid and iso-valeric acid are also produced. The G + C content of the DNA of strain TN-271^T is 48.6 mol %. The type strain is strain TN-271^T and was isolated from the faeces of a healthy adult human. This strain has been deposited in the Japan Collection of Microorganisms (Saitama, Japan) as strain JCM 10539^T.

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