Assignment of Eubacterium sp. VPI 12708 and related strains with high bile acid 7α-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 7α-dehydroxylating activity; the unknown Clostridium-like organisms TN-271T 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-271T and TN-272 formed another single cluster. Clostridium scindens JCM 6567T was the most closely related species for two clusters in the phylogenetic tree. Values for DNA–DNA similarities among C. scindens JCM 6567T, 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 6567T, strain TN-271T and strain N-272 revealed that strains TN-271T and TN-272 were distinct from C. scindens JCM 6567T. On the basis of phylogenetic analysis and DNA–DNA similarity data, it was concluded that strains TN-271T 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-271T (= JCM 10539T).

Keywords: bile acid, 7α-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 7α-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 7α-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 7α-dehydroxylation is an important physiological reaction in the intestinal ecosystem.

Strain VPI 12708, an isolate from human faeces, was reported to exhibit bile acid 7α-dehydroxylating ac-
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 7α-dehydroxylation bacteria and concluded that a few strains were members of the genus *Eubacterium*. Doerner et al. (1997) assessed bile acid 7α-dehydroxylation bacteria for the presence of a *bai*-like gene by Southern hybridization. They found three types of bile acid 7α-dehydroxylation 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 7α-dehydroxylation reaction. The purpose of this study was to examine the taxonomic positions of strain VPI 12708 and other bile acid 7α-dehydroxylation bacteria by means of 16S rDNA sequencing and DNA–DNA similarity experiments.

**METHODS**

**Bacterial strains and cultivation.** Several bacterial strains with known bile acid 7α-dehydroxylation 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, 36B, TN-271 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, H2O2: 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 1000ml distilled water. The Fildes’ solution contained 150 ml physiological saline, 6 ml concentrated HCl, 50 ml horse blood and 1 g peepsin (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 21 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′-dAGAGTTGATCCTGGCAGC-3′ (designated primer 27F) and 5′-dGTTACCTTGTACTAGCTTT-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 5434T (X76163), *Clostridium aminophilum* VPI 14602 (L04165), *Clostridium amiono- valericum* DSM 1283T (X73436), *Clostridium celerecrescens* DSM 5628T (X71848), *Clostridium clostridiforme* ATCC 25537T (M59089), *Clostridium cocoides* DSM 2088 (M59090), *Clostridium nexile* DSM 1787T (X73443), *Clostridium oroticum* ATCC 13619T (M59109), *Clostridium polysaccharolyticum* DSM 1801T (X71858), *Clostridium popul- uleti* ATCC 35295T (X71853), *Clostridium sphenoides* DSM 632T (X73449), *Clostridium symbiosu* ATCC 14940T (M59112), *Clostridium xylanolyticum* ATCC 49623T (X71855), *Coproccocus eutactus* ATCC 27759T (D14148), *Eubacterium cellulosolvens* ATCC 43171T (L34613), *Eubacte- rium formicigerans* ATCC 27755T (L34619), *Eubac- terium limosum* KIST 612 (U67159), *Roseburia cecil* ATCC 33874T (L14467), *Ruminococcus hansenii* ATCC 27752T (D14155), *Ruminococcus productus* ATCC 27340T (L76595) and *Ruminococcus torques* ATCC 27756T (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 Saifu & Miura (1963). DNA base compositions were determined using HPLC (Tamaoka & Komagata, 1984) after enzyme digestion of DNA with deoxyribonuclease. An equimolar mixture of four deoxy-
Table 1. Strains used in this study

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

* 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 6567T 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 6567T.

In strains TN-271T and TN-272, spore formation was observed on EG agar plates. The colony- and cell morphologies of strains TN-271T and TN-272 were very similar to those of C. scindens JCM 6567T (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, 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-271T and TN-272 produced acid from galactose, glucose, raffinose and sucrose, but not from adonitol, amygdalin, cellobiose, dulcitol, erythritol, aesculin, glycerol, glycogen, inositol, inulin, mannitol, melezitose, melibiose, rhamnose, salicin, sorbitol, sorbose, starch or trehalose. For arabinose, fructose, maltose, ribose and xylose, strains

**Fig. 1.** Light micrograph of strain TN-271T showing terminal spores. Cells were cultured on GAM agar. Bar, 10 µm.
Table 2. Characteristics of Clostridium scindens and Clostridium hylemonae

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>C. scindens</th>
<th>C. hylemonae</th>
</tr>
</thead>
<tbody>
<tr>
<td>JCM 6567T</td>
<td>I-10</td>
<td>Y-1113</td>
</tr>
<tr>
<td>Spore formation</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>H₂S production</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acid production from:*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Xylose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ribose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Maltose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*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. hymenonae 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-glucosidase; 4, β-galactosidase; 5, α-glucosidase. All of the strains showed 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-glucosaminidase (–), α-mannosidase (–) and α-fucosidase (–). 

Strains TN-271T and TN-272 showed different sugar-fermentation patterns. With regard to biochemical properties, strains TN-271T and TN-272 did not produce indole, did not reduce nitrate, did not liquefy gelatin and did not hydrolyse aesculin or starch. Strains TN-271T 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 6567T, but strains Y-1113, M18, 36S and TH82 had different patterns of enzyme activity relative to C. scindens JCM 6567T. Strains TN-271T 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 6567T 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 6567T was the most closely related species in the phylogenetic tree. The level of sequence similarity between C. scindens JCM 6567T and strain VPI 12708 was 99.0%.

Strains TN-271T and TN-272 formed a single cluster and C. scindens JCM 6567T was the most closely related species in the phylogenetic tree, but the level of sequence similarity between C. scindens JCM 6567T and strain TN-271T was only 91.3%. The phylogenetic
Bile acid 7α-dehydroxylating clostridia

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

DNA base compositions 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…

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nomic position has not been reported. As in the case of the Member of the genus *Clostridium* (Baron et al., 1980), strain VPI 12708 was identified as a *Clostridium scindens* (Mallonee & Hylemon, 1996; Mallonee et al., 1997; Franklund et al., 1991; Coleman et al., 1990, 1991; Stellwag & Hylemon, 1979; Takamine & Imamura, 1995; White et al., 1980) on faecal bacteria having 7α-dehydroxylating activity have been reported. In these reports, the multistep bile acid 7α-dehydroxylation pathway was studied in detail only for strain VPI 12708 (Baron et al., 1991; Coleman et al., 1990; 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 ascertained. As in the case of strain VPI 12708, other bile acid 7α-dehydroxylating bacteria (strains Y-1113, I-10, M-18, TH-82, 36S, TN-271 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 7α-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 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 6567T on the phylogenetic tree presented in Fig. 2. The similarity level of the 16S rDNA sequences between strain VPI 12708 and *C. scindens* JCM 6567T (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 intergroup DNA similarity values of above 70.0%, indicating that this group was composed of a single species that could be assigned to *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 7α-dehydroxylating activity have been reported. In these reports, the multistep bile acid 7α-dehydroxylation pathway was studied in detail only for strain VPI 12708 (Baron et al., 1991; Coleman et al., 1990; 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 ascertained. As in the case of strain VPI 12708, other bile acid 7α-dehydroxylating bacteria (strains Y-1113, I-10, M-18, TH-82, 36S, TN-271 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 7α-dehydroxylating bacteria remain to be identified in the course of further studies.

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Analysis of the 16S rDNA also showed that strains TN-271T and TN-272 were closely related to *C. scindens* JCM 6567T. However, as shown in Table 4, the levels of reassociation among strains TN-271T, TN-272 and *C. scindens* JCM 6567T demonstrated that TN-271T and TN-272 were clearly distinct from *C. scindens* JCM 6567T and belong to a new species of the genus *Clostridium*, for which we propose the name *Clostridium hylemonae*. The enzymic activities of *x*-galactosidase and *x*-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 7α-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 7α-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-271T and TN-272. Cells are Gram-positive, spore-forming and non-motile. The straight or slightly curved, rod-shaped cells are 1–5·3 x 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 7–5.

Moderate amounts of acetic acid (as the endproduct) are produced in peptone/yeast extract medium supplemented with glucose; small amounts of propionic acid, iso-butyric acid, acetic acid, butyric acid, and lactic acid are also produced. The G+C content of the DNA of strain TN-271T is 48·6 mol %. The type strain is strain TN-271T 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 10539T.

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**REFERENCES**


Saito, H. & Miura, K. (1963). Preparation of transforming...
M. Kitahara and others


