Arch Microbiol (2010) 192:365–372
DOI 10.1007/s00203-010-0566-8

ORIGINAL PAPER

Isolation of a novel bacterium, *Blautia glucerasei* sp. nov., hydrolyzing plant glucosylceramide to ceramide

Hideki Furuya · Yukari Ide · Makiko Hamamoto · Narito Asanuma · Tsuneo Hino

Received: 30 November 2009 / Revised: 3 March 2010 / Accepted: 10 March 2010 / Published online: 31 March 2010
© Springer-Verlag 2010

Abstract A bacterial strain that is capable of hydrolyzing plant glucosylceramide (GluCer) was newly isolated from dog feces. The novel strain, designated as strain HFTH-1^T, hydrolyzed plant GluCer with a variety of chemical structures, but did not hydrolyze glucosylsphingosine, lactosylceramide, or monosialoganglioside GM_3, indicating that strain HFTH-1^T produces GluCer-specific glucosylceramidase. Strain HFTH-1^T was Gram-positive, anaerobic, oval-spore-forming, rod-shaped, lecithinase-negative, and lipase-negative. It fermented a wide variety of carbohydrates and produced mainly acetate, formate, and lactate from glucose. The G + C content of its DNA was 40.7 mol%. The phylogenetic analysis of 16S rRNA sequence revealed that strain HFTH-1^T is placed in the clostridial rRNA cluster XIVa, with *Ruminococcus obeum* as the nearest relative. Pairwise comparison revealed approximately 5.0% sequence divergence between strain HFTH-1^T and the type strain of *R. obeum*. On the basis of its phenotypic characteristics and phylogenetic divergence, it is proposed that the hitherto unknown rod-shaped bacterial strain HFTH-1^T (= DSM 22028^T = NBRC 104932^T) should be placed in the genus *Blautia* as a novel species, *Blautia glucerasei* sp. nov, the only currently known isolate of the species.

Keywords *Blautia glucerasei* sp. nov. · Glucosylceramide · Ceramide · Glucosylceramidase · Intestinal bacteria

Introduction

Glucosylceramide (GluCer) is comprised of a long-chain sphingoid base backbone including sphingosine, an amide-linked fatty acid (FA), and a glucose headgroup. The FA-linked sphingoid is called ceramide. Ceramides and sphingoids are bioactive substances that have been shown to exert various effects on animals (Hannun et al. 1986; Okazaki et al. 1989; Hannun and Linardic 1993; Hannun 1994; Kolesnick and Golde 1994; Ballou et al. 1996; Spiegel and Merrill 1996; Tokura et al. 1996). GluCer and sphingomyelin (SM), precursors of ceramides found in foods (Vesper et al. 1999), are suggested to have both preventive and suppressive effects on colon cancer (Dillehay et al. 1994; Schmelz et al. 1996, 2000; Lemonnier et al. 2003). Ceramides and sphingoids have also been reported to induce apoptosis in cell lines derived from human colon cancer (Ahn and Schroeder 2002). Dietary GluCer and SM are likely to be degraded to ceramides and sphingoids in the intestine, which are then absorbed (Nilsson 1968, 1969; Schmelz et al. 1994). Therefore, prevention and suppression of colon cancer by GluCer and SM may in part be due to apoptosis of colon cells mediated by ceramides and sphingoids.

Communicated by Erko Stackebrandt.

Electronic supplementary material The online version of this article (doi:10.1007/s00203-010-0566-8) contains supplementary material, which is available to authorized users.

H. Furuya · Y. Ide · M. Hamamoto · N. Asanuma · T. Hino (✉)
Department of Life Science, College of Agriculture,
Meiji University, Higashimita, Tama-ku,
Kawasaki 214-8571, Japan
e-mail: hino@isc.meiji.ac.jp

Present Address:
H. Furuya
Department of Pathology and Laboratory Medicine,
Medical University of South Carolina, Charleston,
SC 29425, USA
e-mail: furuya@musc.edu

We reported previously that dietary SM alleviated inflammatory bowel disease (IBD) in mice (Furuya et al. 2008), suggesting that ceramides and/or sphingoids derived from dietary SM alleviate IBD. A similar effect can be expected when GluCer is consumed, but dietary GluCer is barely hydrolyzed to ceramide or sphingoid in the intestinal tract of rats (Nilsson 1969). When various GluCer-containing plant foods were fed to mice, 50–90% of GluCer was excreted in feces (unpublished observations). In vitro and in situ experiments with the ligated intestines of rats showed that virtually no GluCer is absorbed unless it is hydrolyzed to ceramide (unpublished observations). Therefore, enhancement of GluCer hydrolysis by utilizing intestinal bacteria, e.g., as a probiotic, might improve the effect of GluCer.

So far, two GluCer-hydrolyzing bacteria have been isolated from soil. One (*Paenibacillus* sp.) hydrolyzed synthetic GluCer having a short acyl moiety, but the ability to hydrolyze GluCer having a long acyl moiety was low (Sumida et al. 2002). These results suggest that the activity of this bacterium to hydrolyze plant GluCer is low, because the acyl group in plant GluCer is generally long (Bohn et al. 2001). The other (*Rhodococcus* sp.) was also able to hydrolyze GluCer, but the activity was extremely low (Ito and Yamagata 1986, 1989; Ishibashi et al. 2007). Although the traditional culture techniques and the recent development of rRNA sequencing analysis have revealed that intestinal microbiota is highly diverse, no GluCer-hydrolyzing bacterium has been isolated from gut or feces. Thus, it is desirable to find intestinal bacteria that are capable of hydrolyzing plant GluCer, because many plant foodstuffs contain GluCer (Vesper et al. 1999).

This paper reports that a bacterial strain hydrolyzing plant GluCer at a high rate was isolated from dog feces. Based on the morphological, physiological, and biochemical characteristics, and the phylogenetic analysis of the gene sequence from 16S rRNA, the isolate was assigned to a new species in the genus *Blautia*. Recently, the genus *Blautia* was newly proposed, and several species belonging to the genus *Ruminococcus* and *Clostridium* were reclassified into the genus *Blautia* (Liu et al. 2008). The identification of the bacterial isolate and the description of a new species are reported here.

Materials and methods

Bacterial strains and culture conditions

A newly isolated bacterial strain described below (*Blautia glucerasei* sp. nov. HFTH-1^T = DSM 22028^T = NBRC 104932^T) was used for this study. For the isolation of bacteria, a growth medium consisting of clarified ruminal fluid (Ogimoto and Imai 1981) and a basal medium (1:3, v/v) was used. The basal medium contained 0.45 (g/l) of K_2HPO_4, 0.45 of KH_2PO_4, 0.9 of $(NH_4)_2SO_4$, 0.9 of NaCl, 0.12 of $CaCl_2{\cdot}2H_2O$, 0.19 of $MgSO_4{\cdot}7H_2O$, 1.0 of Trypticase-peptone (BBL, Becton–Dickinson, Cockeysville, MD), 1.0 of yeast extract (Difco Laboratories Inc., Detroit, MI), 1.0 of glucose, 1.0 of cellobiose, 1.0 of maltose, and 0.6 of cysteine-HCl (pH 7.0). Growth was estimated by optical density at 600 nm (OD_{600}) of diluted cultures. Peptone-yeast extract-glucose (PYG) medium was used for routine culture of the isolated bacterium.

Isolation of bacteria

As a source of intestinal bacteria, freshly voided feces were collected from 10 animals each of mice, rats, rabbits, pigs, goats, cats, and dogs. The fecal samples were immediately put into vials on ice under a stream of CO_2, frozen at −80°C within a few hours, and subjected to experiments within a month. Feces were suspended in 50 mM potassium phosphate (KPi) buffer (pH 7.0) prepared anaerobically under a stream of CO_2 and inoculated into the growth medium described above containing GluCer (250 mg/l). After overnight incubation, GluCer hydrolysis was estimated. Then, GluCer-hydrolyzing bacteria were isolated from the feces having high GluCer-hydrolyzing activity by the Hungate's role-tube method (Miyazaki et al. 1992) using the above-mentioned growth medium consisting of clarified ruminal fluid and a basal medium (1:3, v/v).

Evaluation of GluCer-hydrolyzing activity

Partially purified GluCer from maize germ was provided by Tsuji Oil Mill co., Ltd. (Matsuzaka, Mie, Japan) and purified to more than 80% purity (most of the remaining 20% consisted of sterylglycoside) by acetone precipitation after hydrolysis with 0.4 N methanolic KOH (38°C, 2 h). Isolated bacteria were cultured at 37°C for 24 h in the growth medium described above, supplemented with 250 mg/l of the purified GluCer. Then, thin-layer chromatography (TLC) was employed for sphingolipid analysis to deal with many samples; i.e., ceramide and sphingoid bases produced from GluCer were separated by TLC and quantified by colorimetry of the sphingoid base as follows. Each culture (3 ml) was shaken vigorously with 6 ml of chloroform–methanol (1:1, v/v; C-M). The chloroform layer separated clearly by centrifugation (1,500 × *g*, 10 min) was harvested, and then concentrated under a stream of N_2. The concentrated lipid fraction was separated by TLC (Silica gel 60, Merck, Darmstadt, Germany) with a developing solvent system of chloroform-acetone (7:3, v/v). The spots corresponding to ceramide and sphingoid (Sigma–Aldrich), judged from the Rf values and sphingolipid-specific colora-

tion (Beiss 1964), were scraped, and put together into a tube. Lipids were extracted with 6 ml of C-M (2:1), dissolved in 1 ml of a solution containing methanol and 16.5% HCl (82:18, v/v), and then hydrolyzed by heating at 100°C for 2 h. After adjustment of the pH to 10–12 with 4 ml of 0.5 N NaOH, the reaction mixture was vigorously shaken with 5 ml of ethyl acetate, and the ethyl acetate layer separated by centrifugation (1,500 × *g*, 10 min) was collected. To the ethyl acetate solution, 2 ml of 10 mM acetate buffer (pH 3.7) and 0.1 ml of 0.5% (w/v) methyl orange were added, and then the mixture was shaken and centrifuged again. The sphingoid–methyl orange complex in the supernatant ethyl acetate phase was measured spectrophotometrically at 415 nm against a blank carried through from the neutralization step (Lauter and Trams 1962).

Hydrolysis of GluCer by a bacterial isolate

A bacterial isolate (strain HFTH-1^T) was grown in 30 ml of PYG medium containing 570 μM of purified maize GluCer (Purity: more than 99%; presented by Tsuji Oil Mill co., Ltd.) at 37°C. Two milliliters of cultures were harvested at different time after inoculation, and GluCer hydrolysis was examined by quantifying sphingolipids. In this case, high-performance liquid chromatography (HPLC) was used to quantify trace amounts of GluCer, ceramide, and sphingoid. After extraction of total lipids with C-M (2:1), the total lipids were dissolved in C-M (4:1, HPLC grade) and filtered through a membrane filter (0.45 μm, Millipore, Billerica, MA). The filtrate (10 μl) was injected into the HPLC system (Shimadzu Co. Ltd, Kyoto, Japan) equipped with a silica gel column (250 mm × 4.6 mm i.d., 5 μm spherical particle size; Shimadzu) and an Evaporative Light Scattering Detector, and eluted with a linear gradient of solvent A (chloroform) and solvent B (methanol + 2% aqueous ammonia, 95:5, v/v). The gradient was from solvents A plus B (9:1) to solvent B.

The activity of GluCer-hydrolyzing enzyme (glucosylceramidase; GluCerase) in cultures was assayed as follows. Strain HFTH-1^T was grown in PYG medium for 8 h (OD_{600} value of 6.8), and cultures were centrifuged (13,000 × *g*, 15 min). The supernatant was adjusted to pH 6.5 with 8% (w/v) Na_2CO_3 and subjected to enzyme assay. The cell pellet was resuspended in the same volume of 50 mM KPi (pH 6.5) before enzyme assay. The assay mixture consisted of 1 ml of 50 mM KPi (pH 6.5) containing 100 μM of GluCer (above 99% purity) and 0.2% (w/v) sodium taurocholate and 1 ml of enzyme sample; i.e., culture supernatant or cell suspension. The assay mixture was incubated at 45°C for 5–10 min, and then ceramide was determined by HPLC as described above. Enzyme activity was expressed as the activity per bacterial cell dry weight, which represents the amount of enzyme produced per cell.

Hydrolysis of different glycosphingolipids by strain HFTH-1^T

Strain HFTH-1^T was grown at 37°C for 10 h in 3 ml of PYG medium containing 200 nmol of soybean GluCer (Over 99% purity, Avanti Polar Lipids, Inc., Alabaster, AL), glucosylsphingosine (GluSph; Avanti Polar Lipids, Inc.), lactosylceramide (LacCer; Avanti Polar Lipids, Inc.), and monosialoganglioside GM_3 (GM_3; HyTest, Ltd., Turku, Finland), respectively. After incubation, ceramide, GluCer, GluSph, LacCer and GM_3 in cultures were quantified by HPLC as described above.

Phylogenetic analysis of the gene sequence from 16S rRNA

The gene from 16S rDNA of strain HFTH-1^T was amplified from the purified genomic DNA by PCR with the bacterial universal primers 27f (5′-AGAGTTTGATCCTGGC TCAG-3′, corresponding to nucleotide position 8 to 27 of *E. coli* 16S rRNA) and 1525r (5′-AAAGGAGGTGATCC AGCC-3′, corresponding to nucleotide position 1543 to 1525 of *E. coli* 16S rRNA), which had been reported by Lane (1991). The amplified product was purified with QIAquick spin PCR purification kit (Qiagen GmbH, Hilden, Germany). The gene sequence was determined with a BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) and the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Sequences were aligned using CLUSTAL X (Version 1.83) software (Thompson et al. 1997) and adjusted manually. *Atopobium minutum* NCFB 2751^T (X67148) was used as the outgroup. Evolutionary distances were calculated with Kimura's two parameter model, and tree was constructed in NEIGHBOR by the neighbor-joining method (Saitou and Nei 1987). The confidence values of branches were determined by performing a bootstrap analysis (Felsenstein 1985) with 1,000 replicates.

The sequence of the gene from 16S rRNA of strain HFTH-1^T was deposited in the GenBank/DDBJ under an accession number of AB439724.

Morphological, physiological, and biochemical characteristics of strain HFTH-1^T

Strain HFTH-1^T was grown in PYG medium at 37°C for 72 h and stored at 4°C for 1 week to check for cellular morphology by Gram staining according to the procedure with Hucker's modifications (Gerhardt 1994). Spore formation was examined by the Wirtz method and tolerance to heat (85°C, 10 min) (Bergey et al. 1984). To examine fermentation products from glucose, strain HFTH-1^T was grown at 37°C for 8 h in the growth medium described above in which maltose and cellobiose were excluded and glucose

was increased to 5.0 g/l (Medium A), and then organic acids were quantified by HPLC as described previously (Hino et al. 1994). The capacity to utilize various carbohydrates was evaluated by growth (OD_{600}) in Medium A in which glucose was replaced by either one of aesculin, arabinose, avicel, cellobiose, fructo-oligosaccharide, fructose, galactose, inulin, lactose, maltose, mannitol, mannose, pectin, potato starch, raffinose, sorbitol, sucrose, xylose, and xylan (3 g/l).

The G + C content in DNA was determined by HPLC after digestion of isolated DNA with P1 nuclease and alkaline phosphatase (Kamlage et al. 1997). The amino acid compositions in cell wall peptidoglycan were analyzed by HPLC (Waters, MA) as described previously (Takahashi et al. 1989), and the peptidoglycan type was determined as described by Schleifer and Kandler (1972). Cellular fatty acids were extracted and analyzed according to the standard protocol of the Sherlock Microbial Identification System Version 6 (MIDI, DE).

Activities of nineteen enzymes were assayed by the API ZYM (bioMérieux, Lyon, France) according to the manufacturer's instructions. Lecithinase and lipase activities were assayed as follows. Cells were grown in PYG medium supplemented with lecithin (phosphatidylcholine; PC) or triolein (as triglyceride; TG) at 37°C for 24 h, and total lipids were extracted from each culture as described above. The lipids were separated by TLC using the developing solvent system of chloroform–methanol-H_2O-acetate (65:25:4:1, v/v) for PC analysis, or C-M (95:5, v/v) for TG analysis. Spots were detected by spraying 50% sulfuric acid and heating at 120°C for 15 min. The TLC plates were photographed by Personal Density Scanning Imager (Molecular Dynamics, Sunnyvale, CA), and the amounts of PC and TG were estimated by densitometry using ImageQuaNT (Molecular Dynamics, Sunnyvale, CA).

Results

Isolation of GluCer-hydrolyzing bacteria

When feces from 10 animals each of mice, rats, rabbits, pigs, goats, cats, and dogs were incubated overnight with GluCer, high GluCer-hydrolyzing activity was found only in one canine feces. Two different GluCer-hydrolyzing bacterial strains were isolated from more than 600 colonies prepared from the canine feces. Of the two isolates, one showed nearly three times higher GluCer-hydrolyzing activity than the other for both maize germ GluCer and soybean GluCer (data not shown). The isolate having higher activity was designated as strain HFTH-1^T and used for the experiments described below.

Hydrolysis of glycosphingolipids in growing cultures of strain HFTH-1^T

When strain HFTH-1^T was grown in GluCer-containing PYG medium, GluCer was readily hydrolyzed, and virtually 100% of GluCer was converted to ceramide in 11 h (Fig. 1). No sphingoid, a degradation product of ceramide, was detected, indicating that strain HFTH-1^T does not degrade ceramide. Approximately 80% of GluCerase activity was found in the culture supernatant (2.3 nmol/min/mg of cell dry weight), whereas nearly 20% activity was in the cell fraction (0.6 nmol/min/mg of cell dry weight). Strain HFTH-1^T was unable to hydrolyze GluSph, LacCer, or GM_3 (data not shown).

Identification of strain HFTH-1^T

To establish the phylogenetic position of strain HFTH-1^T, comparative 16S rRNA gene sequence analysis was performed, and almost the entire sequence of the 16S rRNA gene (1,468 bp) was determined. The similarity values of the 16S rRNA gene sequence between strain HFTH-1^T and its closely related species (*Ruminococcus obeum* and seven species of the genus *Blautia*) within the rRNA cluster XIVa were 92–95%. The highest level of 16S rRNA gene similarity with strain HFTH-1^T was 95.3% of the type strain of *R. obeum* ATCC 29174^T. The sequence diversity between strain HFTH-1^T and the nearest relative, the type strain *R. obeum* was 3.0%. The sequence data for strain HFTH-1^T was aligned with 32 sets of published data. The 16S rRNA gene sequence (1,160 nt; excluding incomparable nucleotides and spaces) was used for analysis. A phylogenetic tree showed that strain HFTH-1^T formed a coherent cluster with *R. obeum*, *Blautia luti*, *Blautia wexlerae*, *Blautia schinkii*, *Blautia hansenii*, *Blautia producta*, *Blautia coccoides*, and *Blautia hydrogenotrophica* in the clostridial rRNA cluster XIVa (Collins et al. 1994), that was statistically well supported (bootstrap = 89%) (Fig. 2). The major branching

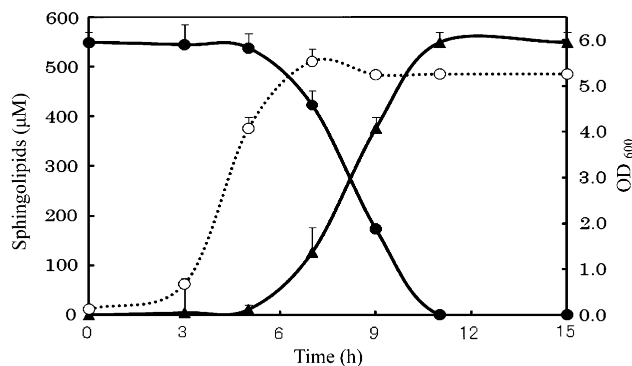

Fig. 1 Hydrolysis of GluCer by strain HFTH-1^T. *Bars* indicate SD ($n = 2$). *Symbols* indicate growth (*open circle*), GluCer (*filled circle*), and ceramide

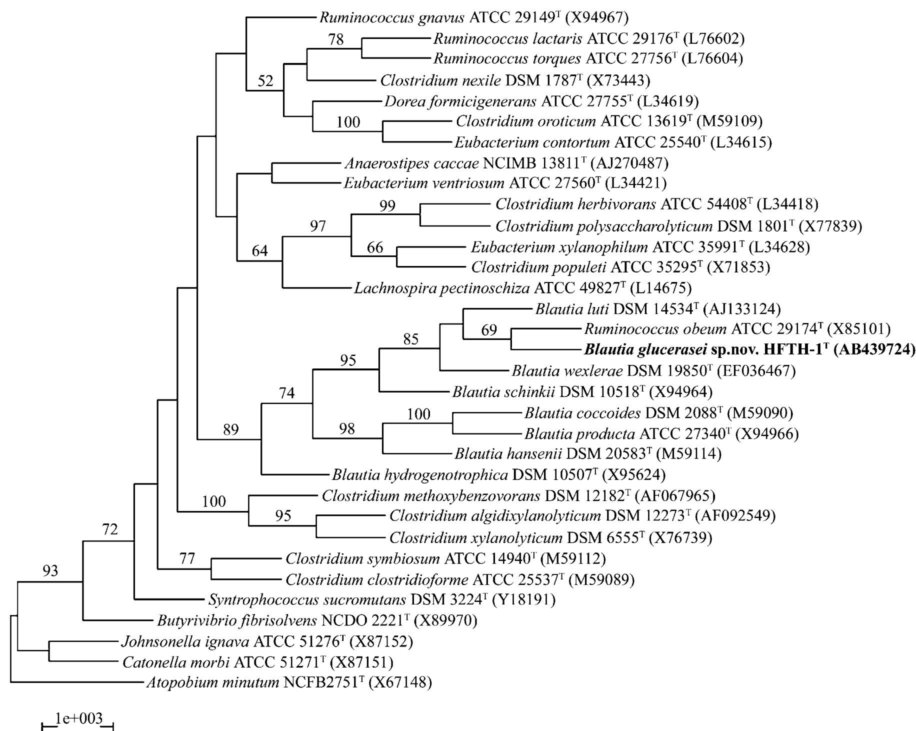

Fig. 2 Phylogenetic tree showing the inter-relationships of Blautia glucerasei sp. nov. HFTH-1^T with some of its nearest relatives within the rRNA cluster XIVa. The tree was constructed by the neighbor-joining method as described in the text. *The numbers at nodes* indicate bootstrap values greater than 50% (1000 replicates). *Bar* 1e+003 substitutions per nucleotide position.

orders were confirmed by using the maximum composite likelihood method (Online Resource 1) (Tamura et al. 2007).

The 16S rRNA gene sequence analysis showed that the closely phylogenetic relative to strain HFTH-1^T is *R. obeum.* However, strain HFTH-1^T was easily distinguished from *R. obeum* based on several phenotypic characteristics. In contrast to *R. obeum,* strain HFTH-1^T formed spores and produced β-N-acetylglucosaminidase, α-fructosidase, and α-mannosidase. The characteristics that differentiate strain HFTH-1^T from its three most closely related species in the clostridial rRNA cluster XIVa (Fig. 2 and Online Resource 1) are summarized in Table 1.

Table 1 Salient characteristics of an isolated bacterium (HFTH-1^T) and the closely related species in the clostridial rRNA cluster XIV$_a$

Characteristics[a]	HFTH-1^T	*R.obeum*[b]	*B. luti*[b]	*B. wexlerae*[b]
Spore formation	+	−	−	−
Acid produced from				
Cellobiose	+	−	+	±
Mannitol	−	−	−	±
Mannose	−	+	+	+
Sorbitol	−	+	+	±
Enzyme activity				
β-N-acetylglucosaminidase	+	−	+	−
Alkaline phosphatase	−	+	+	−
α-Fructosidase	+	−	+	+
α-Glucosidase	−	+	+	+
α-Mannosidase	+	−	+	−
Fermentation products from glucose[c]	A, F, L	A	A, S	A, S

[a] + positive, − negative, ± different among strains

[b] Data refer to the type strains apart from *B. wexlerae*. Data are from references (Kaneuchi et al. 1976; Bergey et al. 1984; Bernalier et al. 1996; Simmering et al. 2002; Liu et al. 2008)

[c] A, F, L, and S represent acetate, formate, lactate, and succinate, respectively. The amounts of these acids produced were in this order

Discussion

A bacterial strain (HFTH-1^T) that is capable of hydrolyzing GluCer to ceramide at high rate was newly isolated from canine feces. Strain HFTH-1^T hydrolyzed GluCer, but not GluSph, LacCer, or GM_3, indicating that the strain has GluCerase that specifically hydrolyzes GluCer. Nearly 80% of GluCerase activity was present in the culture supernatant, and the remaining 20% of GluCerase was considered to be bound to the bacterial cell surface. This result indicates that GluCerase is secreted out of cells. It is conceivable that GluCer is hydrolyzed by extracellular GluCerase in strain HFTH-1^T. However, physiological implications of GluCer-hydrolyzing activity in strain HFTH-1^T are unknown at present.

As mentioned in the introduction, *Paenibacillus* sp. strain TS12 isolated from soil produced β-glucosidase, which hydrolyzed synthetic GluCer (the FA moiety was C12:0, and the sphingoid moiety was sphingosine), but the hydrolysis rate decreased with the increase in the length of

the acyl moiety (Sumida et al. 2002). In contrast, strain HFTH-1^T readily hydrolyzed GluCer extracted from soybean and maize germ, which contain C14–C24 FAs as the acyl moiety and various sphingoids as the sphingoid moiety; e.g., sphingosine, 4-hydroxy-trans-8-sphingenine, trans-4, trans-8-Sphingadienine, and trans-4, cis-8-Sphingadienine. Since all the plant GluCer known so far contain similar FAs and sphingoids (Bohn et al. 2001), it is conceivable that strain HFTH-1^T hydrolyzes most plant GluCer at high rates.

It has also been reported that *Rhodococcus* sp. isolated from soil was able to hydrolyze GluCer, but the reported activity is inferred to be far lower than the activity of strain HFTH-1^T (Ito and Yamagata 1986). This implies that strain HFTH-1^T is the first example of a GluCer-hydrolyzing microbe that can be put to practical use. Because strain HFTH-1^T is an intestinal bacterium and showed no acute toxicity in mouse tests (unpublished observations), strain HFTH-1^T could be used as a probiotic to augment the conversion of dietary GluCer to ceramide that is much more readily absorbed from the intestine (Nilsson 1968, 1969; Schmelz et al. 1994). When strain HFTH-1^T is orally administered, its spores may survive in the stomach, germinate in the small intestine, and grow in the large intestine, as reported for *Clostridium butyricum* (Sato and Tanaka 1997). Thus, administration of strain HFTH-1^T as a probiotic might be effective for health.

On the other hand, ceramide is the major component of the stratum corneum (SC) (Lampe et al. 1983a, b) and hence has attracted attention to its effect on skin disorders; e.g., dry skin, psoriasis, and atopic dermatitis. It has been reported that the ceramide content in SC is decreased in patients with atopic dermatitis (Melnik et al. 1988; Imokawa et al. 1991; Macheleidt et al. 2002) and psoriasis (Motta et al. 1993, 1995), suggesting that a deficiency of ceramide is involved in these diseases. Thus, to prevent and alleviate skin lesions, it may be desirable to increase ceramide in intercellular lipids among SC corneocytes by percutaneous ceramide administration. However, because no means is available at present to produce ceramide at low cost, GluCer is used as a ceramide source for cosmetics and supplements. Strain HFTH-1^T hydrolyzes GluCer to ceramide exclusively, and does not degrade ceramide further. It is presumed that ceramide is more effective than GluCer, and therefore, strain HFTH-1^T or its GluCerase may be useful for the industrial production of ceramide from plant GluCer at low cost. GluCer can be obtained from inedible parts or residues of grains and fruits.

The phylogenetic tree inferred from the gene sequence of 16S rRNA suggested that strain HFTH-1^T is related to the members in the genus of *Blautia* (Fig. 2 and Online Resource 1). Because the genus *Blautia* is proposed and reclassified on the basis of comparative 16S rRNA sequencing and phylogenetic studies (Liu et al. 2008), these data indicate that strain HFTH-1^T is affiliated with the genus *Blautia*. Strain HFTH-1^T has the highest 16S rRNA similarity to the type strain of *R. obeum* (95.3%), but *R. obeum* does not form spores. Morphological, physiological, and biochemical characteristics obviously discriminate strain HFTH-1^T from the related species in the genus of *Blautia*. In conclusion, these data confirmed that strain HFTH-1^T represents a novel species in the genus *Blautia* described as *Blautia glucerasei*.

Description of *Blautia glucerasei* sp. nov

Blautia glucerasei (glu.cera.sei. N. L. gen. n. *glucerasei* of GluCerase, having GluCerase). Cells are Gram-positive, motile, strictly anaerobic, lecithinase- and lipase-negative, oval-spore-forming rods, 0.8–1.0 µm in diameter and 2.3–3.0 µm in length. Colonies on PYG agar appear white and circular with a smooth surface and edges (1–1.5 mm in diameter after 1 day of growth). Cells grow well in liquid or solid PYG medium under anaerobic conditions. The temperature range for growth is 25–45°C with optimum growth at 37°C. The optimal initial pH is 7.0. Cells possess a DNA G + C content of 40.7%. Acid is produced from glucose, maltose, galactose, fructose, lactose, inulin, raffinose, arabinose, xylose, xylan, cellobiose, starch, pectin, and fructo-oligosaccharide. The products of glucose fermentation are acetate, formate, and lactate. Cell wall peptidoglycan type of Schleifer and Kandler is A1γ (Schleifer and Kandler 1972). Cellular long-chain fatty acids are of the straight-chain saturated and monounsaturated types, with $C_{16:0}$ dimethyl acetal acids (*ca.* 26%), $C_{12:0}$ (*ca.* 19%) and $C_{16:0}$ (*ca.* 12%) predominating, and $C_{14:0}$ (*ca.* 9%), $C_{18:0}$ dimethyl acetal acids (*ca.* 7%), $C_{16:0}$ aldehyde (*ca.* 5%), $C_{11:0}$ dimethyl acetal acids (*ca.* 5%), $C_{14:0}$ dimethyl acetal acids (*ca.* 4%), $C_{18:0}$ (*ca.* 3%), and $C_{18:1}$ w9c dimethyl acetal acids (*ca.* 1%) as the minor components (representing more than 1.00%). Cells possess naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, leucine allyl amidase, acid phosphatase, β-glucosidases, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase, but do not possess alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), valine allyl amidase, cystine allyl amidase, trypsin, α-chymotrypsin, β-glucuronidase, or α-glucosidases. GluCer is hydrolyzed to ceramide.

The type strain is HFTH-1^T (= DSM 22028^T = NBRC 104932^T), which was isolated from feces of a healthy dog, in Japan.

Acknowledgments This study was supported in part by a Grant-in-Aid for Scientific Research (No. 18780205 and 18580274) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT), and by "High-Tech Research Center" Project for Private Universities: matching fund subsidy from MEXT, 2006–2008.

We express our thanks to Tsuji Oil Mill co., Ltd. (Matsuzaka, Mie, Japan) for providing purified GluCer.

References

Ahn EH, Schroeder JJ (2002) Sphingoid bases and ceramide induce apoptosis in HT-29 and HCT-116 human colon cancer cells. Exp Biol Med (Maywood) 227:345–353

Ballou LR, Laulederkind SJ, Rosloniec EF, Raghow R (1996) Ceramide signalling and the immune response. Biochim Biophys Acta 1301:273–287

Beiss U (1964) On the Paper Chromatographic Separation of Plant Lipids. J Chromatogr 13:104–110

Bergey DH, Krieg NR, Holt JG (1984) Bergey's manual of systematic bacteriology. Williams & Wilkins, Baltimore

Bernalier A, Willems A, Leclerc M, Rochet V, Collins MD (1996) Ruminococcus hydrogenotrophicus sp. nov., a new H2/CO2-utilizing acetogenic bacterium isolated from human feces. Arch Microbiol 166:176–183

Bohn M, Heinz E, Luthje S (2001) Lipid composition and fluidity of plasma membranes isolated from corn (Zea mays L.) roots. Arch Biochem Biophys 387:35–40

Collins MD et al (1994) The phylogeny of the genus Clostridium: proposal of five new genera and eleven new species combinations. Int J Syst Bacteriol 44:812–826

Dillehay DL, Webb SK, Schmelz EM, Merrill AH Jr (1994) Dietary sphingomyelin inhibits 1, 2-dimethylhydrazine-induced colon cancer in CF1 mice. J Nutr 124:615–620

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Furuya H, Ohkawara S, Nagashima K, Asanuma N, Hino T (2008) Dietary sphingomyelin alleviates experimental inflammatory bowel disease in mice. Int J Vitam Nutr Res 78:41–49

Gerhardt P (1994) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, D.C

Hannun YA (1994) The sphingomyelin cycle and the second messenger function of ceramide. J Biol Chem 269:3125–3128

Hannun YA, Linardic CM (1993) Sphingolipid breakdown products: anti-proliferative and tumor-suppressor lipids. Biochim Biophys Acta 1154:223–236

Hannun YA, Loomis CR, Merrill AH Jr, Bell RM (1986) Sphingosine inhibition of protein kinase C activity and of phorbol dibutyrate binding in vitro and in human platelets. J Biol Chem 261:12604–12609

Hino T, Shimada K, Maruyama T (1994) Substrate Preference in a Strain of Megasphaera elsdenii, a Ruminal Bacterium, and Its Implications in Propionate Production and Growth Competition. Appl Environ Microbiol 60:1827–1831

Imokawa G, Abe A, Jin K, Higaki Y, Kawashima M, Hidano A (1991) Decreased level of ceramides in stratum corneum of atopic dermatitis: an etiologic factor in atopic dry skin? J Invest Dermatol 96:523–526

Ishibashi Y et al (2007) A novel endoglycoceramidase hydrolyzes oligogalactosylceramides to produce galactooligosaccharides and ceramides. J Biol Chem 282:11386–11396

Ito M, Yamagata T (1986) A novel glycosphingolipid-degrading enzyme cleaves the linkage between the oligosaccharide and ceramide of neutral and acidic glycosphingolipids. J Biol Chem 261:14278–14282

Ito M, Yamagata T (1989) Purification and characterization of glycosphingolipid-specific endoglycosidases (endoglycoceramidases) from a mutant strain of Rhodococcus sp. Evidence for three molecular species of endoglycoceramidase with different specificities. J Biol Chem 264:9510–9519

Kamlage B, Gruhl B, Blaut M (1997) Isolation and characterization of two new homoacetogenic hydrogen-utilizing bacteria from the human intestinal tract that are closely related to Clostridium coccoides. Appl Environ Microbiol 63:1732–1738

Kaneuchi C, Benno Y, Mitsuoka T (1976) Clostridium coccoides, a New Species from the Feces of Mice. Int J Syst Bacteriol 26:482–486

Kolesnick R, Golde DW (1994) The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. Cell 77:325–328

Lampe MA et al (1983a) Human stratum corneum lipids: characterization and regional variations. J Lipid Res 24:120–130

Lampe MA, Williams ML, Elias PM (1983b) Human epidermal lipids: characterization and modulations during differentiation. J Lipid Res 24:131–140

Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt EaG M (ed) Nucleic acid techniques in bacterial systematics. John Wiley and Sons, Chichester, pp 115–175

Lauter CJ, Trams EG (1962) Spectrophotometric determination of sphingosine. J Lipid Res 3:136

Lemonnier LA, Dillehay DL, Vespremi MJ, Abrams J, Brody E, Schmelz EM (2003) Sphingomyelin in the suppression of colon tumors: prevention versus intervention. Arch Biochem Biophys 419:129–138

Liu C, Finegold SM, Song Y, Lawson PA (2008) Reclassification of Clostridium coccoides, Ruminococcus hansenii, Ruminococcus hydrogenotrophicus, Ruminococcus luti, Ruminococcus productus and Ruminococcus schinkii as Blautia coccoides gen. nov., comb. nov., Blautia hansenii comb. nov., Blautia hydrogenotrophica comb. nov., Blautia luti comb. nov., Blautia producta comb. nov., Blautia schinkii comb. nov. and description of Blautia wexlerae sp. nov., isolated from human faeces. Int J Syst Evol Microbiol 58:1896–1902

Macheleidt O, Kaiser HW, Sandhoff K (2002) Deficiency of epidermal protein-bound omega-hydroxyceramides in atopic dermatitis. J Invest Dermatol 119:166–173

Melnik B, Hollmann J, Plewig G (1988) Decreased stratum corneum ceramides in atopic individuals–a pathobiochemical factor in xerosis? Br J Dermatol 119:547–549

Miyazaki K, Hino T, Itabashi H (1992) Effects of extracellular pH on the intracellular pH and membrane potential of cellulolytic ruminal bacteria, Ruminococcus albus, Ruminococcus flavefaciens, and Fibrobacter succinogenes. J Gen Appl Microbiol 38:567–573

Motta S, Monti M, Sesana S, Caputo R, Carelli S, Ghidoni R (1993) Ceramide composition of the psoriatic scale. Biochim Biophys Acta 1182:147–151

Motta S, Sesana S, Ghidoni R, Monti M (1995) Content of the different lipid classes in psoriatic scale. Arch Dermatol Res 287:691–694

Nilsson A (1968) Metabolism of sphingomyelin in the intestinal tract of the rat. Biochim Biophys Acta 164:575–584

Nilsson A (1969) Metabolism of cerebroside in the intestinal tract of the rat. Biochim Biophys Acta 187:113–121

Ogimoto K, Imai S (1981) Atlas of Rumen Microbiology. Japan Scientific Societies Press, Tokyo, Japan

Okazaki T, Bell RM, Hannun YA (1989) Sphingomyelin turnover induced by vitamin D3 in HL-60 cells. Role in cell differentiation. J Biol Chem 264:19076–19080

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sato R, Tanaka M (1997) Intestinal distribution and intraluminal localization of orally administered Clostridium butyricum in rats. Microbiol Immunol 41:665–671

Schleifer KH, Kandler O (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol Rev 36:407–477

Schmelz EM, Crall KJ, Larocque R, Dillehay DL, Merrill AH Jr (1994) Uptake and metabolism of sphingolipids in isolated intestinal loops of mice. J Nutr 124:702–712

Schmelz EM, Dillehay DL, Webb SK, Reiter A, Adams J, Merrill AH Jr (1996) Sphingomyelin consumption suppresses aberrant colonic crypt foci and increases the proportion of adenomas versus adenocarcinomas in CF1 mice treated with 1, 2-dimethylhydrazine: implications for dietary sphingolipids and colon carcinogenesis. Cancer Res 56:4936–4941

Schmelz EM, Sullards MC, Dillehay DL, Merrill AH Jr (2000) Colonic cell proliferation and aberrant crypt foci formation are inhibited by dairy glycosphingolipids in 1, 2-dimethylhydrazine-treated CF1 mice. J Nutr 130:522–527

Simmering R et al (2002) *Ruminococcus luti* sp. nov., isolated from a human faecal sample. Syst Appl Microbiol 25:189–193

Spiegel S, Merrill AH Jr (1996) Sphingolipid metabolism and cell growth regulation. FASEB J 10:1388–1397

Sumida T, Sueyoshi N, Ito M (2002) Molecular cloning and characterization of a novel glucocerebrosidase of Paenibacillus sp. TS12. J Biochem 132:237–243

Takahashi Y, Iwai Y, Tomoda H, Nimura N, Kinoshita T, Omura S (1989) Optical resolution of 2, 6-diaminopimelic acid stereoisomers by high performance liquid chromatography for the chemotaxonomy of actinomycete strains. J Gen Appl Microbiol 35:27–32

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Tokura Y, Wakita H, Yagi H, Nishimura K, Furukawa F, Takigawa M (1996) Th2 suppressor cells are more susceptible to sphingosine than Th1 cells in murine contact photosensitivity. J Invest Dermatol 107:34–40

Vesper H, Schmelz EM, Nikolova-Karakashian MN, Dillehay DL, Lynch DV, Merrill AH Jr (1999) Sphingolipids in food and the emerging importance of sphingolipids to nutrition. J Nutr 129:1239–1250