[0065] [Table 1]

Registration name	Strain name	NCBI accession No.	Identity (%)
Bacillus sp. (in: firmicutes)	SKF111034	MZ229683.1	96.07
Bacillus sp. JAMB750	JAMB750	AB128830.1	94.21
Bacillus suaedae	YZJH907-2	NR_181548.1	94.08
Bacillus suaedae	YZJH907-2	NW291641.1	94.08
Alkalihalobacillus alcalophilus	SLA-270	MT125838.1	94.08
uncultured bacterium	−	HG764498.1	94.02
Halalkalibacterium halodurans	−	CP040441.1	94.02
Halalkalibacterium halodurans	−	AF542087.1	94.02
Bacillus sp. C-3	−	AB043847.1	94.02
Fermentibacillus polygoni	IEB14	LC054228.1	93.94
Fermentibacillus polygoni	IEB3	NR_149287.1	93.94

[0066] (4) Production of Phylogenetic Tree

Next, a phylogenetic tree based on partial base sequences of 16S rRNAs was produced using the results of the homology search in Example 1(3) above, and the phylogenetic position of the komham196 was analyzed. The phylogenetic tree was produced using MEGA (Molecular Evolutionary Genetics Analysis) software, which is a molecular evolution phylogenetic analysis tool, and the neighbor-joining method. FIG. 1 shows the results.

[0067] FIG. 1 shows a phylogenetic tree of the komham196 based on partial base sequences of 16S rRNAs. In FIG. 1, a scale bar on the left lower side indicates 0.050, meaning a 5% base difference. A numeral located at a branch point of a phylogenetic branch indicates a bootstrap value.

[0068] (5) Examination of Taxonomical Properties 1

For the komham196, morphological observation and a test for physiological properties (also referred to as a "bacterial first-stage test" below) were conducted, and the taxonomical properties were examined. Specifically, the komham196 was aerobically cultured at 50°C for 24 hours. After the culture, catalase reaction, oxidase reaction, acid/gas production from glucose, and oxidation/fermentation (O/F) of glucose were tested based on the morphological observation using an optical microscope and the method described in Reference 4. FIG. 2 and Table 2 show the results.

Reference 4: Barrow GI, Feltham RKA. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd edition. Cambridge: University Press; 1993.

[0077] [Table 4]

No.	Enzyme	Substrate	pH	Result	
			8.5	Positive	Negative
1	Negative control			Colorless or original color of sample	
2	Alkaline phosphatase	2-Naphthyl phosphite	8.5	Purple	Colorless or light yellow
3	Esterase (C4)	2-Naphthyl butyrate	6.5	Purple	
4	Esterase-lipase (C8)	2-Naphthyl caprylate	7.5	Purple	
5	Lipase (C14)	2-Naphthyl myristate	7.5	Purple	
6	Leucine aryl amidase	L-leucyl-2-naphthyl amide	7.5	Orange	
7	Valine aryl amidase	L-valyl-2-naphthyl amide	7.5	Orange	
8	Cystine aryl amidase	L-cystyl-2-naphthyl amide	7.5	Orange	
9	Trypsin	N-benzoyl-DL-arginine-2-naphthyl amide	8.5	Orange	
10	α-Chymotrypsin	N-glutaryl-phenylalanine-2-naphthyl amide	7.5	Orange	
11	Acid phosphatase	2-Naphthyl phosphate	5.4	Purple	
12	Naphthol-AS-BI-phosphohydrase	Naphthol-AS-BI-phosphate	5.4	Blue	
13	α-Galactosidase	6-Br-2-naphthyl-α-D-galactopyranoside	5.4	Purple	
14	β-Galactosidase	2-Naphthyl-β-D-galactopyranoside	5.4	Purple	
15	β-Glucuronidase	Naphthol-AS-BI-β-D-glucuronide	5.4	Blue	
16	α-Glucosidase	2-Naphthyl-α-D-glucopyranoside	5.4	Purple	
17	β-Glucosidase	6-Br-2-naphthyl-β-D-glucopyranoside	5.4	Purple	
18	N-acetyl-β-glucosaminidase	1-Naphthyl-N-acetyl-β-D-glucosamide	5.4	Brown	
19	α-Mannosidase	6-BR-2-naphthyl-α-mannopyranoside	5.4	Purple	
20	α-Fucosidase	2-Naphthyl-α-L-fucopyranoside	5.4	Purple	

[0078] FIG. 5 is a photograph illustrating the results of the bacterial second-stage test using API (registered trademark) ZYM. Table 4 above is a table showing the results of the bacterial second-stage test. As shown in FIG. 5 and Table 4 above, it was found that the komham196 has esterase activity, esterase-lipase activity, and naphthol-AS-BI-phosphohydrolase activity.

[0079] (8) Morphological Observation

Morphological observation of the komham196 was conducted. Specifically, morphological observation of the komham196 was conducted using an electron microscope. The komham196 was cultured using a PYA (pH 9) liquid medium for use for the observation using a transmission electron microscope (TEM). After the culture, a droplet of the suspension was placed on a carbon membrane, and negative staining was conducted using 2% uranyl acetate. After the staining, the observation was conducted at a voltage of 100 kV using a TEM (model: JEM-1400 Flash; manufactured by JEOL Ltd.). Also, the komham196 was cultured using a PYA (pH 9) liquid medium for use for the observation using a scanning electron microscope (SEM). After the culture, prefixation was conducted using 2% glutaraldehyde. After the prefixation, postfixation was conducted using 2% osmium tetraoxide. After the postfixation, washing was conducted using 0.1 mol/l phosphate buffer. After the washing,

dehydration was conducted using ethanol at serial concentrations (30% to 100%). After the dehydration, the samples were dried through critical point drying. After the drying, the samples were coated with osmium. Thereafter, the observation was conducted at a voltage of 5 kV using a SEM (model: JSM-7500F; manufactured by JEOL Ltd.; ×20,000). FIG. 6 shows the results.

[0080] FIG. 6 shows photographs illustrating the results of the morphological observation of the komham196 using an electron microscope. FIG. 6(A) is a photograph illustrating the result of the morphological observation of the komham196 using the SEM, and FIG. 6(B) is a photograph illustrating the result of the morphological observation of the komham196 using the TEM. The scale bar in FIG. 6 indicates 1 μm. As shown in FIG. 6, it was found that the komham196 is a bacillus having a flagellum.

[0081] (9) Determination of Base Sequence of Genomic DNA

The base sequence of the genomic DNA of the komham196 was determined. Specifically, first, the genomic DNA of the komham196 was extracted. Genomictip 20G (manufactured by QIAGEN N.V.) was used for the extraction. After the extraction, a library was prepared using SMRTbell Express Template Prep Kit 2.0 (manufactured by PacBio) in accordance with the procedure described in Procedure Checklist-Preparing HiFi Libraries from Low DNA Input Using SMRTbell (registered trademark) Express Template Prep Kit 2.0. After the preparation, Binding kit 2.2 (manufactured by PacBio) was used to form a polymerase complex of the library. After the formation, sequencing was conducted using Sequel IIe (manufactured by PacBio). Thereafter, SMRT (registered trademark) Link (v. 11.0.0.146107) was used to remove the adaptor sequence from the obtained sequence, and thus subreads were produced. After the production, a consensus sequence was produced through alignment of the subreads. After the production, a consensus sequence in which the average quality value per read was less than 20 was removed, and thus HiFi reads were produced. After the production, a HiFi read of 1000 bases or less was deleted using Filtlong (ver. 0.2.1). After the deletion, high-quality HiFi reads were assembled using default conditions of Flye (ver. 2.9-b1774). As a result, it was found that the genomic DNA of the komham196 consists of the base sequence 2.

[0082] (10) ANI Analysis of Genome Base Sequence

An ANI analysis was further conducted in order to confirm that the komham196 was a new species of microorganisms. Specifically, pyani v0.2.11 and BLAST were used (Reference 7) to determine the ANI value of the base sequence obtained in Example 1(1) above. FIG. 7 shows the results.

Reference 7: Pritchard, Leighton, et al. "Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens." Analytical Methods 8.1 (2016): 12-24.

[0087] FIG. 8 is a graph illustrating the results of the proliferation test. As shown in FIG. 8, it was found that in the sample to which chitin, starch, or casein had been added, the DNA level increased after 48 hours compared with that before the start of the culture (0 hours). From these results, it could be confirmed that the komham196 has an ability to utilize chitin, starch, and casein.

[0088] As described above, the present disclosure has been described with reference to the embodiments and the example, but the present disclosure is not limited to the above-described embodiments and example. Various modifications that can be understood by a person skilled in the art can be made in the configurations and details of the present disclosure without departing from the scope of the present disclosure.

[0089] The present application claims the benefit of priority from Japanese Patent Application No. 2023-055613 filed on March 30, 2023, the entire disclosure of which is incorporated herein.

[0090] <Supplementary Notes>

Some or all of the embodiments and the examples given above can be described as in the following supplementary notes, but the scope of the present disclosure is not limited thereto.

(Supplementary Note 1)

A microorganism

belonging to the family Bacillaceae, and

deposited with the accession number NITE BP-03807.

(Supplementary Note 2)

A microorganism that is a mutant strain of the microorganism according to Supplementary Note 1.

(Supplementary Note 3)

The microorganism according to Supplementary Note 2,

wherein the mutant strain includes a gene having a base sequence of (R) below as a 16S rRNA gene:

(R) a base sequence of (R1), (R2), or (R3) below:

(R1) a base sequence represented by SEQ ID NO: 1;

(R2) a base sequence consisting of the base sequence represented by SEQ ID NO: 1 with deletion, substitution, insertion, and/or addition of 1 to 58 bases;

(R3) a base sequence having 96% or more identity to the base sequence represented by SEQ ID NO: 1.

(Supplementary Note 4)

The microorganism according to Supplementary Note 2 or 3,

wherein the mutant strain has at least one property selected from the group consisting of

CLAIMS

1. A microorganism
belonging to the family Bacillaceae, and
deposited with the accession number NITE BP-03807.

2. A microorganism that is a mutant strain of the microorganism according to claim 1.

3. The microorganism according to claim 2,
wherein the mutant strain includes a gene having a base sequence of (R) below as a 16S rRNA gene:
(R) a base sequence of (R1), (R2), or (R3) below:
(R1) a base sequence represented by SEQ ID NO: 1;
(R2) a base sequence consisting of the base sequence represented by SEQ ID NO: 1 with deletion, substitution, insertion, and/or addition of 1 to 58 bases;
(R3) a base sequence having 96% or more identity to the base sequence represented by SEQ ID NO: 1.

4. The microorganism according to claim 2 or 3,
wherein the mutant strain has at least one property selected from the group consisting of properties (1) to (7) and (8) below:
(1) being a Gram-positive spore-forming bacillus;
(2) growing at a temperature of 30°C to 55°C;
(3) growing at a pH of 9 to 10;
(4) being positive for catalase reaction;
(5) being positive for oxidase reaction;
(6) having activity to metabolize D-arabinose, D-ribose, D-xylose, L-xylose, D-fructose, L-sorbose, amygdalin, D-maltose, D-turanose, D-lyxose, D-tagatose, potassium 2-ketogluconate, and/or potassium 5-ketogluconate;
(7) having activity to metabolize esculin, lysine hydrochloride, and/or gelatin;
(8) having esterase activity, lysine decarboxylase activity, gelatinase activity, esterase-lipase activity, and/or naphthol-AS-BI-phosphohydrolase activity.

5. The microorganism according to claim 4, further having a property (9) below:
(9) having an ability to utilize chitin, starch, and/or casein.

6. The microorganism according to any one of claims 2 to 5, wherein the mutant strain includes a genomic DNA having an ANI value of 74% or more relative to a base sequence represented by SEQ ID NO: 2.

7. A microorganism
belonging to the family Bacillaceae, and
including a gene having a base sequence of (R) below as a 16S rRNA gene:
(R) a base sequence of (R1), (R2), or (R3) below:
(R1) a base sequence represented by SEQ ID NO: 1;
(R2) a base sequence consisting of the base sequence represented by SEQ ID NO: 1 with deletion, substitution, insertion, and/or addition of 1 to 58 bases;
(R3) a base sequence having 96% or more identity to the base sequence represented by SEQ ID NO: 1.

8. The microorganism according to claim 7, having at least one property selected from the group consisting of properties (1) to (8) and (9) below:
(1) being a Gram-positive spore-forming bacillus;
(2) growing at a temperature of 30°C to 55°C;
(3) growing at a pH of 9 to 10;
(4) being positive for catalase reaction;
(5) being positive for oxidase reaction;
(6) having activity to metabolize D-arabinose, D-ribose, D-xylose, L-xylose, D-fructose, L-sorbose, amygdalin, D-maltose, D-turanose, D-lyxose, D-tagatose, potassium 2-ketogluconate, and/or potassium 5-ketogluconate;
(7) having activity to metabolize esculin, lysine hydrochloride, and/or gelatin;
(8) having esterase activity, lysine decarboxylase activity, gelatinase activity, esterase-lipase activity, and/or naphthol-AS-BI-phosphohydrolase activity;
(9) having an ability to utilize chitin, starch, and/or casein.

9. A microorganism
belonging to the family Bacillaceae, and
having properties (1) to (9) below:
(1) being a Gram-positive spore-forming bacillus;
(2) growing at a temperature of 30°C to 55°C;
(3) growing at a pH of 9 to 10;

(4) being positive for catalase reaction;

(5) being positive for oxidase reaction;

(6) having activity to metabolize D-arabinose, D-ribose, D-xylose, L-xylose, D-fructose, L-sorbose, amygdalin, D-maltose, D-turanose, D-lyxose, D-tagatose, potassium 2-ketogluconate, and/or potassium 5-ketogluconate;

(7) having activity to metabolize esculin, lysine hydrochloride, and/or gelatin;

(8) having esterase activity, lysine decarboxylase activity, gelatinase activity, esterase-lipase activity, and/or naphthol-AS-BI-phosphohydrolase activity;

(9) having an ability to utilize chitin, starch, and/or casein.

10. A composition for use in degradation of garbage, including the microorganism according to any one of claims 1 to 9.

11. A garbage treatment method comprising a step of bringing the microorganism according to any one of claims 1 to 9 into contact with garbage to allow the microorganism to degrade the garbage.

12. The treatment method according to claim 11 or 12, wherein the degradation is conducted at a temperature of 30°C to 55°C.

13. The treatment method according to claim 11 or 12, wherein the degradation is conducted at a pH of 9 to 10.

14. The treatment method according to any one of claims 11 to 13, wherein the garbage is selected from the group consisting of crustacean exoskeletons, fish, meat, and rice.