

DESCRIPTION

TITLE OF INVENTION

MICROORGANISM, COMPOSITION FOR USE IN DEGRADATION OF GARBAGE, AND
GARBAGE TREATMENT METHOD

TECHNICAL FIELD

[0001] The present disclosure relates to a microorganism, a composition for use in degradation of garbage, and a garbage treatment method.

BACKGROUND ART

[0002] Conventionally, wastes such as garbage have been incinerated in an incineration plant. However, in recent years, garbage treatment and the like such as composting of garbage through microbial treatment have been attracting attention for the purpose of reducing environmental load.

[0003] As the garbage, exoskeletons of crustaceans such as crabs and shrimps are disposed in large quantities. However, the exoskeletons contain insoluble chitin having a strong crystal structure and are thus difficult to dispose (Non-Patent Literature 1). Accordingly, various ways such as disposal of the exoskeletons through microbial treatment have been searched.

Citation List

Non-Patent Literature

[0004] Non-Patent Literature 1: Kazuto Seki, et al., "Composting Fishery Wastes with Wood Meal (1): Chemical property of the waste and the early stage of the degradation process by a composting machine under cold environment." (2007): 8-14.

SUMMARY OF INVENTION

Technical Problem

[0005] Accordingly, it is an object of the present disclosure to, for example, provide a novel microorganism capable of degrading polysaccharides such as chitin and starch, and/or proteins such as casein, etc.

Solution to Problem

[0006] To achieve the aforementioned object, a microorganism of the present disclosure belongs to the family Bacillaceae, and

is deposited with the accession number NITE BP-03807.

[0007] The microorganism of the present disclosure belongs to the family Bacillaceae, and 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:

5 (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.

10 [0008] The microorganism of the present disclosure belongs to the family Bacillaceae, and has 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;

15 (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;

20 (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.

[0009] A composition for use in degradation of garbage of the present disclosure includes the microorganism of the present disclosure.

[0010] A garbage treatment method of the present disclosure includes a step of bringing the microorganism of the present disclosure into contact with garbage to allow the microorganism to degrade the garbage.

30 Advantageous Effects of Invention

[0011] With the present disclosure, it is possible to, for example, provide a novel microorganism capable of degrading polysaccharides such as chitin and starch, and/or proteins such as casein, etc.

35 Brief Description of Drawings

[0012] [FIG. 1] FIG. 1 shows a phylogenetic tree of komham196 based on partial base sequences of 16S rRNAs in Example 1.

[FIG. 2] FIG. 2 is a photograph illustrating the results of morphological observation of komham196 using an optical microscope in Example 1.

5 [FIG. 3] FIG. 3 is a photograph illustrating the results of the bacterial second-stage test using API (registered trademark) 50CH in Example 1.

[FIG. 4] FIG. 4 is a photograph illustrating the results of the bacterial second-stage test using API (registered trademark) 20E and API (registered trademark) 20NE in Example 1.

10 [FIG. 5] FIG. 5 is a photograph illustrating the results of the bacterial second-stage test using API (registered trademark) ZYM in Example 1.

[FIG. 6] FIG. 6 shows photographs illustrating the results of morphological observation of komham196 using an electron microscope in Example 1.

[FIG. 7] FIG. 7 shows the results of an ANI analysis in Example 1.

[FIG. 8] FIG. 8 is a graph illustrating the results of a proliferation test in Example 1.

15

DESCRIPTION OF EMBODIMENTS

[0013] The following specifically describes examples of the present disclosure. The descriptions of the other disclosures can be applied to each disclosure below unless otherwise stated.

20 [0014] As a result of extensive research, the inventors of the present invention found a novel microorganism, isolated and identified the microorganism, and thus established the present disclosure. Specifically, it was confirmed from the results of the morphological observation, the test for physiological and biochemical properties, the analysis of the partial sequence of 16S rRNA, and the ANI analysis of the genome sequence that the microorganism of the present
25 disclosure belongs to the family Bacillaceae, but it was found that it does not belong to the known genera belonging to the family Bacillaceae and is a novel microorganism. Furthermore, as a result of extensive research, the inventors of the present invention found that the microorganism has an ability to degrade chitin, starch, and/or casein contained in crustacean exoskeletons, and the like, and thus established the present disclosure. Accordingly, it is
30 expected that the microorganism of the present disclosure will be used for degradation and the like of crustacean exoskeletons, fish, meat, and/or rice, etc.

[0015] The microorganism of the present disclosure is a new species of microorganisms that belongs to the family Bacillaceae. The microorganism of the present disclosure includes a bacterial strain deposited with the accession number NITE BP-03807 or a progeny line thereof
35 (also referred to as "Alkalithermobacillus komhamensis strain" or "komham196" hereinafter).

[0016] <komham196>

The komham196 or a progeny line thereof of the present disclosure has, for example, properties (1) to (8) below.

(1) Being a Gram-positive spore-forming bacillus;

(2) growing at a temperature of 30°C to 55°C and preferably 40°C to 50°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.

[0017] The komham196 of the present disclosure may have any one, some, or all of the properties (1) to (8) above.

[0018] The properties (1) to (8) above can be measured in conformity with items (5) to (8) of Example 1, which will be described below.

[0019] The komham196 of the present disclosure may further have, for example, a property (9) below.

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

[0020] An example of the komham196 of the present disclosure is a bacterial strain deposited with the accession number NITE BP-03807 (deposited line) or a progeny line thereof. The following shows the information on the deposit.

Deposit Type: International Deposit

Name of Deposit Institute: Patent Organism Depositary of the National Institute of Technology and Evaluation

Address: #120, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba 2920818, Japan

(komham196)

Accession Number: NITE BP-03807

Identification: komham196

Receipt Date: January 23, 2023

[0021] The komham196 of the present disclosure includes, for example, a gene having the base sequence of (R) below as the 16S rRNA gene, and preferably has a gene having the base

sequence of (R1) below, namely the 16S rRNA gene having the base sequence represented by SEQ ID NO: 1.

[0022] (R) A base sequence of (R1), (R2), or (R3) below:

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

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

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

[0023] In (R1) above, the base sequence of SEQ ID NO: 1 is the base sequence encoding 16S RNA. The base sequence of SEQ ID NO: 1 above can be isolated from, for example, the komham196.

[0024] Base Sequence of 16S rRNA Gene of Komham196 (SEQ ID NO: 1)

5'-

AATGCGGCGTGCCTAATACATGCAAGTCGAACGAACCGACGAGGAGCTTGCTCCTCT
 15 TAGGTTAGTGGCGGATGGGTGAGTAACACGTGGGCAACCTGCCCTGTAGATTGGGAT
 AACTTACGGAAACGTGAGCTAATACCGAATAATCCTTTTAGACGCATGTCTAAAAGTT
 AAAAGATGGCTCTGCTATCACTACAGGATGGGCCCCGCGGCGCATTAGCTAGTTGGTAA
 GGTAACGGCTTACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCAC
 ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCC
 20 GCAATGGACGCAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCCTTCGGGT
 CGTAAAACTCTGTTGTTAGGGAAGAACAAGTATCGGAGTAACTGCCGGTACCTTGAC
 GGTACCTAACGAGGAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
 GtGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGGGCGCGCAGGCGGTCTCTTAAGT
 CTGATGTGAAATCTTGCGGCTCAACCGCAAGCGGTCATTGGAACTGGGAGACTTGA
 25 GTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGG
 AGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAAGTACGCTGAGGCGCGAA
 AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGT
 GCTAGGTGTTAGGGGTTTCGATGCCCTTAGTGCCGAAGTAAACACATTAAGCACTCCG
 CCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGGCCCGCACA
 30 AGCAGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGA
 CATCCTTTGACCACCCTAGAGATAGGGCTTTCCCTTCGGGGGACAAAGTGACAGGT
 GGTGCATGGTTGTCGTCAGCTCGTGTCTGTGAGATGTTGGGTAAAGTCCCGCAACGAG
 CGCAACCCTTGACCTTAGTTGCCAGCATTGAGTTGGGCACTTTAAGGTGACTGCCGGT
 GACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGG
 35 CTACACACGTGCTACAATGGATGGTACAGAGGGTCGCGAAGCCGCGAGGCGGAGCC

AATCCCACAAAGCCATTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGC
 CGGAATTGCTAGTAATCGCGGATCAGAATGCCGCGGTGAATACGTTCCCGGGCCTTGT
 ACACACCGCCCGTCACACCACGAGAGTTTGTAAACACCCGAAGTCGGTGAGGTAACA
 CACTACGTGAGCCAGCCGCCTAAGGGGACAAG-3'

- 5 [0025] The "one or several" bases in (R2) above may be any number of bases as long as, for example, a microorganism having the 16S rRNA gene that includes the base sequence of (R2) above maintains the properties of the komham196 above. The properties of the komham196 above may be, for example, the properties (1) to (9) above, and preferably the property (9) above (the same applies hereinafter). The "one or several" bases in (R2) above refer to, for example, 10 to 58 bases, 1 to 43 bases, 1 to 29 bases, 1 to 14 bases, 1 to 10 bases, 1 to 5 bases, 1 to 3 bases, 1 or 2 bases, or 1 base, in the base sequence of (R1) above. In the present disclosure, the numerical range of the number of bases or the like discloses, for example, all positive integers belonging to that range. Specifically, for example, "1 to 5" means disclosure of all of "1, 2, 3, 4, and 5" (the same applies hereinafter).
- 15 [0026] The "identity" in (R3) above may refer to any degree of identity as long as, for example, a microorganism having the 16S rRNA gene that includes the base sequence of (R3) above maintains the properties of the komham196 above. In (R3) above, the "identity" to the base sequence of (R1) above is, for example, 96% or more, 97% or more, 98% or more, or 99% or more. The "identity" can be determined through alignment of two base sequences or two amino acid sequences (the same applies hereinafter). The above-mentioned alignment can be 20 calculated using, for example, BLAST, FASTA, or the like with default parameters.
- [0027] The 16S rRNA gene can be measured using, for example, a known method. As the known method, the PCR (Polymerase Chain Reaction) method, sequencing analysis, or the like can be used. rRNAs including the 16S rRNA are present in all living organisms except viruses 25 and are important molecules involved in protein synthesis. Accordingly, it is known that the rates of evolution of the rRNAs are relatively low, and the rRNAs have high homology to one another at a level of species. When the DNA-DNA hybridization homology of a bacterium to a reference strain is 70% or more, they are defined as being the same species (Reference 1). Meanwhile, in the bacterial species identification based on the 16S rRNA sequence, a homology 30 of 97% or more indicates a close relationship, and a homology of 98.7% or more indicates a high possibility of being the same species (Reference 2). Accordingly, if there is no species to which the homology of a bacterium is 98.7% or more in the bacterial species identification based on the 16S rRNA sequence, it is determined that the bacterium is a new species. Specifically, the bacterial species identification is conducted by comparing a target bacterium with the base 35 sequence of the 16S rRNA registered in a public gene bank. Examples of the public gene bank

include DNA Data Bank of Japan, GenBank, EMBL, and the like. The comparison of the known base sequences can be conducted using, for example, a base sequence analysis software (e.g., BLAST).

Reference 1: Wayne L. G., Brenner D. J., Colwell R. R., Grimont P. A. D., Kandler O., Krichevsky M. I., Moore L. H., Moore W. E. C., Murray R. G. E., Stackebrandt E., Starr M. P., Truper H. G. 1987; Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. *Int. J. Syst. Bacteriol.* 37: 463-464

Reference 2: Stackebrandt, Erko. "Taxonomic parameters revisited: tarnished gold standards." *Microbiol. Today* 33 (2006): 152-155.

[0028] The identity of the 16S rRNA of the komham196 of the present invention to the base sequence of the 16S rRNA gene of a known microorganism is preferably, for example, less than 98.7%, 98% or less, 97% or less, 96.07% or less, 96% or less, 95% or less, or 90% or less, and furthermore, the homology is preferably 95.99% or less. Examples of the known microorganism include *Bacillus* sp. (in: firmicutes), *Bacillus* sp. JAMB750, *Bacillus suaedae*, *Alkalihalobacillus alcalophilus*, *Halalkalibacterium halodurans*, and the like.

[0029] Examples of closely-related species of the komham196 of the present disclosure include bacteria belonging to the genus *Bacillus*.

[0030] The komham196 of the present disclosure has, for example, the genomic DNA having the base sequence represented by SEQ ID NO: 2.

[0031] For example, ANI (Average Nucleotide Identity) analysis may be used to identify the bacterial species based on the genomic DNA of a bacterial strain. The ANI analysis is a method for determining whether species are the same or different by calculating the identity (ANI value) between full-length genome sequences or draft genome sequences of a target strain and a comparative strain on a computer. In the ANI analysis, when the value is 95% or more, it is possible to determine that the genomes belong to the same bacterial species, and when the value is less than 95%, it is possible to determine that the genomes belong to different bacterial species. Regarding a method of conducting the ANI analysis, for example, Reference 3 below can be referred to.

Reference 3: Rodriguez-R, Luis M., and Konstantinos T. Konstantinidis. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. No. e1900v1. *PeerJ Preprints*, 2016.

[0032] The ANI value of the genome sequence of the komham196 of the present disclosure (SEQ ID NO: 2) to the genome sequence of a known microorganism is preferably, for example, less than 95%, less than 90%, less than 85%, less than 80%, or less than 75%. Examples of the known microorganism include *Paenibacillus macerans*, *Alkalihalobacillus alcalophilus*,

Salipaludibacillus agaradhaerens, Sporosarcina pasteurii, Alkalihalobacillus clausii, Evansella clarkii, Alkalihalobacillus krulwichiae, Alkalihalobacillus gibsonii, Alkarihalophilus pseudofirmus, Halalkalibacterium halodurans, and the like.

[0033] It is preferable that the komham196 of the present disclosure includes the genomic DNA with, for example, the ANI value to the base sequence represented by SEQ ID NO: 2 of 74% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more.

[0034] The komham196 of the present disclosure includes, for example, a gene having the base sequence of (G) below as the genomic DNA, and preferably has a gene having the base sequence of (G1) below, namely the genomic DNA having the base sequence represented by SEQ ID NO: 2.

[0035] (G) A base sequence of (G1), (G2), or (G3) below:

(G1) a base sequence represented by SEQ ID NO: 2;

(G2) a base sequence consisting of the base sequence represented by SEQ ID NO: 2 with deletion, substitution, insertion, and/or addition of one or several bases;

(G3) a base sequence having 80% or more identity to the base sequence represented by SEQ ID NO: 2.

[0036] In (G1) above, the base sequence of SEQ ID NO: 2 is the base sequence encoding the genomic DNA. The base sequence of SEQ ID NO: 2 above can be isolated from, for example, the komham196.

[0037] The "one or several" bases in (G2) above may be any number of bases as long as, for example, a microorganism that includes the genomic DNA having the base sequence of (G2) above maintains the properties of the komham196 above. The properties of the komham196 above may be, for example, the properties (1) to (9) above, and preferably the property (9) above (the same applies hereinafter). The "one or several" bases in (G2) above refer to, for example, 1 to 587773 bases, 1 to 440829 bases, 1 to 293886 bases, 1 to 146942 bases, 1 to 117553 bases, 1 to 88165 bases, 1 to 58776 bases, 1 to 29387 bases, 1 to 14694 bases, 1 to 7347 bases, 1 to 3673 bases, or 1 to 1836 bases in the base sequence of (G1) above.

[0038] The "identity" in (G3) above may refer to any degree of identity as long as, for example, a microorganism that includes the genomic DNA having the base sequence of (G3) above maintains the properties of the komham196 above. In (G3) above, the "identity" to the base sequence of (G1) above is, for example, 80% or more, 85% or more, 90% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more.

[0039] The taxon to which the komham196 of the present disclosure belongs may be identified through, for example, the bacterial morphological observation, the bacterial first-stage test, the

bacterial second-stage test, and the like. Examples of the bacterial morphological observation include observations of the color tone of colonies, the cellular morphology, the Gram-stainability, the spore forming ability, and the like. Examples of the bacterial first-stage test include observations of the properties of colonies, the cellular morphology, and the motility, and tests for physiological and biochemical properties such as the catalase test, the oxidase test, and the O/F test, and the like. Examples of the bacterial second-stage test include tests for an ability to utilize a carbon source, oxidizability/fermentability, enzyme activity, and the like using a kit for a test for physiological and biochemical properties. The komham196 of the present disclosure exhibits, for example, the morphological characteristics and the physiological and biochemical properties shown in the results of the bacterial morphological observation, the bacterial first-stage test, the bacterial second-stage test, and the like in Example 1, which will be described below.

[0040] The komham196 of the present disclosure can be passaged through, for example, culture conducted in conformity with the culture conditions in Example 1, which will be described below. Thus, in the present disclosure, progeny lines of the komham196 can be obtained.

[0041] <Mutant Strain>

The microorganism of the present disclosure may be a mutant strain of the komham196 or a progeny line thereof.

[0042] The mutant strain of the present disclosure is, for example, a bacterium that maintains the taxonomical properties as a new species of the komham196. The properties above may be, for example, the properties (1) to (9) above. The mutant strain may have, for example, any one, some, or all of the properties (1) to (9) above. The description of the komham196 of the present disclosure can be applied to the mutant strain of the present disclosure.

[0043] The mutant strain of the present disclosure can be acquired through, for example, mutagenesis in the komham196, introduction of an exogenous gene into the komham196, or the like. The mutagenesis can be caused by, for example, introducing mutation using an ordinary method. A method for introducing the mutation can be a method such as homologous recombination or a genome-editing technology in which ZFN, TALEN, CRISPR-CAS9, CRISPR-CPF1, or the like is used. The method for introducing the mutation may be, for example, a mutagenesis such as a site-directed mutagenesis. The method for introducing the mutation may be, for example, a random mutagenesis. Examples of the random mutagenesis include: irradiation with α -rays, β -rays, γ -rays, X-rays, or the like; chemical treatment with a mutagenic agent such as ethyl methanesulfonate (EMS) or ethylnitrosourea (ENU); heavy ion beam treatment; and the like.

[0044] The mutant strain includes a gene having the base sequence of (R) below as the 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

5 with deletion, substitution, insertion, and/or addition of one or several bases;

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

[0045] The descriptions of (R1) to (R3) for the komham196 of the present disclosure can be applied to (R1) to (R3) above.

10 [0046] The mutant strain includes, for example, a gene having the base sequence of (G) below as the genomic DNA, and preferably has a gene having the base sequence of (G1) below, namely the genomic DNA having the base sequence represented by SEQ ID NO: 2.

[0047] (G) A base sequence of (G1), (G2), or (G3) below:

(G1) a base sequence represented by SEQ ID NO: 2;

15 (G2) a base sequence consisting of the base sequence represented by SEQ ID NO: 2 with deletion, substitution, insertion, and/or addition of one or several bases;

(G3) a base sequence having 80% or more identity to the base sequence represented by SEQ ID NO: 2.

[0048] The descriptions of (G1) to (G3) for the komham196 of the present disclosure can be applied to (G1) to (G3) above.

[0049] <Garbage Treatment Method>

In another example of the present disclosure, provided is a method with which garbage can be treated. In a garbage treatment method (also referred to as a "treatment method" hereinafter) of the present disclosure, the microorganism of the present disclosure is used. The treatment method of the present disclosure includes a step (also referred to as a "degradation step" hereinafter) of bringing the microorganism of the present disclosure into contact with garbage to allow the microorganism to degrade the garbage. The treatment method of the present disclosure is characterized by using the microorganism of the present disclosure, and there is no particular limitation on configurations and conditions other than this.

25 [0050] The term "garbage" as used herein means food waste. Examples of the food waste include leavings of edible portions of food, inedible portions of food that are not eaten from common sense in common eating habits, and the like. Examples of the inedible portions include shells of fish and shellfish, internal organs and bones of fish and meat, vegetable waste, and the like. In the present disclosure, the garbage to be treated in the degradation step need
35 only contain garbage, and may contain other wastes. Examples of the other wastes include

paper such as wrapping paper, a plastic bag, plastic wrap, a rubber glove, and the like.

[0051] In the degradation step, the microorganism and the garbage can be brought into contact with each other by, for example, mixing them. The mixing can be mechanically conducted using, for example, an agitator.

5 [0052] In the degradation step, the temperature (degradation temperature) at which the degradation is conducted is, for example, a temperature at which the microorganism of the present disclosure can treat the garbage. The degradation temperature is, for example, 30°C to 55°C, and preferably 40°C to 50°C.

10 [0053] In the degradation step, the pH (degradation pH) at which the degradation is conducted is, for example, a pH at which the microorganism of the present disclosure can treat the garbage. The degradation pH is, for example, pH 9 to 10.

[0054] In the degradation step, for example, oxygen may be supplied. The oxygen can be supplied by, for example, agitating a mixture of the garbage and the microorganism of the present disclosure. The oxygen is supplied at an interval of, for example, 1 hour to 10 hours,
15 preferably 5 hours to 8 hours, and more preferably 8 hours. The oxygen is supplied, for example, for 1 minute to 30 minutes, preferably 1 minute to 10 minutes, and more preferably 5 minutes.

[0055] In the degradation step, the water content in the mixture of the garbage and the microorganism of the present disclosure is, for example, 40% to 70%, preferably 50% to 70%,
20 and more preferably 55% to 65%.

[0056] It is preferable that the garbage contains, for example, a polysaccharide and/or a protein. Examples of the polysaccharide include chitin, starch, and the like. Examples of the protein include casein and the like.

[0057] Examples of the garbage include crustacean exoskeletons, fish, meat, rice, and the like.
25

Examples

[0058] The following describes examples of the present invention. However, the present invention is not limited to the examples below. Commercially available reagents were used based on their protocols unless otherwise stated. Note that "mol/l" may also be referred to as
30 "M".

[0059] [Example 1]

The novel microorganism of the present disclosure could be identified and utilize chitin, starch, and casein added to compost.

[0060] (1) Isolation of Komham196

35 Compost from a fish and shellfish waste disposal plant in Hokkaido, Japan, was

acquired as a sample. A PYA medium (pH 9.0) was prepared by dissolving 8 g of hipectone, 3 g of yeast extract, 1 g of dipotassium phosphate, and 0.25 g of ammonium chloride in 900 ml of distilled water and adding 100 ml of alkaline buffer (sodium carbonate, pH 9.0) after the dissolution. After the preparation, the sample was diluted with distilled water and then
 5 inoculated into the PYA medium. After the inoculation, culture was conducted at 50°C. After the culture, colonies that had grown on the medium were separated. After the separation, colonies were inoculated on the PYA culture medium, and culture was repeated. Thereafter, the novel microorganism, komham196, was isolated through isolation and purification.

[0061] (2) Determination of 16S rRNA Base Sequence of komham196

10 To identify the microorganism, the base sequence of the 16S rRNA of the komham196 isolated in Example 1(1) was analyzed. Specifically, DNA was extracted from the isolated komham196. After the extraction, the following primer set (manufactured by FASMAC; synthesis requested) and KOD One (registered trademark) PCR Master Mix (manufactured by TOYOBIO Co., Ltd.) were added to the DNA to prepare a reaction solution. After the
 15 preparation, the 16S rRNA gene in the DNA was amplified using a thermal cycler (T100 Thermal Cycler, manufactured by Bio-Rad Laboratories, Inc.). After the amplification, DNA sequencing was conducted using the obtained PCR product, and the base sequence of the 16S rRNA of the komham196 isolated in Example 1(1) above was obtained. The obtained base sequence was the base sequence represented by SEQ ID NO: 1.

20 [0062] - 27F primer (SEQ ID NO: 3)

5'-AGAGTTTGATCMTGGCTCAG-3'

- 1492R primer (SEQ ID NO: 4)

5'-GGYTACCTTGTTACGACTT-3'

[0063] (3) Comparison with 16S rRNA Base Sequence of Existing Microorganisms

25 The 16S rRNA base sequence obtained in Example 1(2) above was used to compare the 16S rRNA of the komham196 with the 16S rRNAs of existing microorganisms. Specifically, the comparison was conducted using BLAST. Homology search of BLAST was used to search microorganisms having a sequence that is the same as or similar to the 16S rRNA base sequence of the komham196. Table 1 below shows the search results. As shown in Table 1 below, it
 30 was found that the SKF111034 strain of the genus *Bacillus* (*Bacillus* sp.), which is a microorganism having the base sequence represented by SEQ ID NO: 5, has the 16S rRNA base sequence closest to the 16S rRNA base sequence of the komham196. The percent homology (identity) between the 16S rRNA of the komham196 and the 16S rRNA base sequence of the SKF111034 strain was 96.07%, which was lower than 98.7%. It was found from these results
 35 that no microorganisms have 98.7% or more identity to the komham196, and thus the

komham196 is a new species.

[0064] Base Sequence of 16S rRNA Gene of SKF111034 Strain (SEQ ID NO: 5)

5'-

CTGGGCGGGGTGCCTATACATGCAGTCGAGCGAACAGAAGAGGAGCTTGCTCCTCGG
 5 ACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCCTGTAGATGGGGATA
 ACTCCGGGAAACCGGGGCTAATACCGAATGAATCTTTCAAACGCATGTTTGAAAGCT
 AAAAGATGGTTATGCTATCACTACAGGATGGGCCCCGCGGCGCATTAGCTAGTTGGTAA
 GGTAACGGCTTACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCAC
 ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCC
 10 GCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCCTTCGGGT
 CGTAAAACTCTGTTGTTAGGGAAGAACAAGTACGAGAGTCACTGCTCGTACCTTGAC
 GGTACCTAACGAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG
 GTGGCAAGCGTTGTCCGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCTTTTAAG
 TCTGATGTGAAATCTTGCGGCTCAACCGTAAGCGGTCATTGGAAACTGGGAGACTTG
 15 AGTACAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTG
 GAGGAACACCAGTGCGGAAGGCGACTCTCTGGTCTGTAAGTACGCTGAGGCGCGA
 AAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAG
 TGCTAGGTGTTAGGGGTTTCGATGCCCTTAGTGCCGAAGTCAACACATTAAGCACTCC
 GCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGGCCCGCAC
 20 AAGCAGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTG
 ACATCCTTTGACCACCCTAGAGATAGGGCTTTCCCCTTCGGGGGACAAAGTGACAGG
 TGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTAAAGTCCCGCAACGA
 GCGCAACCCTTGACCTTAGTTGCCAGCATTGAGTTGGGCACTCTAAGGTGACTGCCG
 GTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTG
 25 GGCTACACACGTGCTACAATGGATGGTACAGAGGGCAGCAAAACCGCGAGGTCGAG
 CGAATCCCATAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAG
 CCGGAATTGCTAGTAATCGCGGATCAGAATGCCGCGGTGAATACGTTCCCGGGCCTTG
 TACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACC
 ATAGGAGCCAGCCGCCCTAAGGGTGAACAGAT-3'

30

[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 an offspring evolution phylogenetic analysis tool, and the neighbor-joining method. FIG. 1 shows the results.

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.

[0069] [Table 2]

Test item		komham196
Culture temperature (°C)		50
Cell morphology		Bacillus (0.8 to 0.9 × 1.0 to 4.0 μm)
Presence or absence of spores		+
Motility		+
Colony morphology	Culture medium	PYA agar medium
	Culture time (hr)	24
	Diameter	1 mm or less
	Color tone	Cream color
	Shape	Circular
	Elevation	Convex
	Margin	Entire margin
	Surface texture and the like	Smooth
	Degree of transparency	Translucent
	Degree of thickness	Butter-like
Growth temperature test	30 (°C)	+
	37 (°C)	+
	40 (°C)	+
	50 (°C)	+
	55 (°C)	+
Growth pH test	pH 9	+
	pH 10	+
Catalase reaction		+
Oxidase reaction		+
Acid/gas production from glucose (Acid production / gas production)		NT
O/F test (oxidation / fermentation)		NT

+: positive, -: negative

NT: The test was conducted, but the culture medium used was not suitable for the growth of the specimen, resulting in no data and results.

5

[0070] FIG. 2 is a photograph illustrating the result of the morphological observation of the komham196 using an optical microscope. As a result of the morphological observation using an optical microscope, it was found that komham196 form colonies as shown in FIG. 2.

[0071] As shown in Table 2 above, it was found that the komham196 is a bacillus with motility, forms spores, is positive for catalase reaction, and is positive for oxidase reaction.

10

[0072] (6) Examination of Taxonomical Properties 2

In order to determine whether the komham196 was a gram-positive bacteria or a gram-negative bacteria, a test for classification and identification was conducted. Specifically, a test for classification and identification using 3% KOH was conducted based on References 5 and 6 below. The komham196 was cultured using the PYA (pH 9) liquid medium. Within 24 hours from the culture, bacteria were picked up from the plate culture using a sterilized toothpick. After being picked up, the bacteria were mixed with one drop of 3% KOH dropped on a glass slide. After the mixing, the bacteria were observed using an optical microscope (BX50F4, manufactured by Olympus Corporation). In the observation, bacteria that formed sticky threads were determined as gram-negative bacteria, whereas bacteria that further melted thereafter into a

15

20

mobile liquid were determined as gram-positive bacteria. As a result, it was not confirmed that a mixture of the bacteria and 3% KOH formed sticky threads. It was found from these results that the komham196 is a gram-positive bacterium.

Reference 5: Ryu, E. I. H. Y. O. "A simple method of differentiation between Gram-positive and Gram-negative organisms without staining." *Kitasato Archives of Experimental Medicine* 17 (1940): 58-63.

Reference 6: Shigeru Takeda. "Identification of Bacteria in Activated Sludge -A Simple Testing Method Comparison-" *Japanese Journal of Water Treatment Biology*, 31.3 (1995): 161-167.

10 [0073] (7) Examination of Taxonomical Properties 3

For the komham196, a test for biochemical properties (also referred to as a "bacterial second-stage test" below) was conducted, and the taxonomical properties were examined. Specifically, in the bacterial second-stage test, API (registered trademark) 50CH, API (registered trademark) 20E, API (registered trademark) 20NE, and API (registered trademark) ZYM (all of these were manufactured by bioMérieux S.A.) were used. FIGS. 3 to 5 and Tables 3 and 4 show the results.

[0074] [Table 3]

No.	Substrate component			No.	Substrate component		
0	Control			25	ESC	Esculin Ferric citrate	
1	GLY	Glycerol		26	SAL	Salicin	
2	ERY	Erythritol		27	CEL	D-cellobiose	
3	DARA	D-arabinose	+	28	MAL	D-maltose	+
4	LARA	L-arabinose		29	LAC	D-lactose (bovine-derived)	
5	RIB	D-ribose	+	30	MEL	D-melibiose	
6	DXYL	D-xylose	+	31	SAC	D-saccharose (sucrose)	
7	LXYL	L-xylose	+	32	TRE	D-trehalose	
8	ADO	D-adonitol		33	INU	Inulin	
9	MDX	Methyl- β -D-xylopyranoside		34	MLZ	D-melezitose	
10	GAL	D-galactose		35	RAF	D-raffinose	
11	GLU	D-glucose		36	AMD	Starch (amidon)	
12	FRU	D-fructose	+	37	GLYG	Glycogen	
13	MNE	D-mannose		38	XLT	Xylitol	
14	SBE	L-sorbose	+	39	GEN	Gentiobiose	
15	RHA	L-rhamnose		40	TUR	D-turanose	+
16	DUL	Dulcitol		41	LYX	D-lyxose	+
17	INO	Inositol		42	TAG	D-tagatose	+
18	MAN	D-mannitol		43	DFUC	D-fucose	
19	SOR	D-sorbitol		44	LFUC	L-fucose	
20	MDM	Methyl- α -D-mannopyranoside		45	DARL	D-arabitol	
21	MDG	Methyl- α -D-glucopyranoside		46	LARL	L-arabitol	
22	NAG	N-acetylglucosamine		47	GNT	Potassium gluconate	
23	AMY	Amygdalin	+	48	2KG	Potassium 2-ketogluconate	+
24	ARB	Arbutin		49	5KG	Potassium 5-ketogluconate	+

[0075] FIG. 3 is a photograph illustrating the results of the bacterial second-stage test using API (registered trademark) 50CH. Table 3 above is a table showing the results of the bacterial second-stage test. As shown in FIG. 3 and Table 3 above, it was found that the komham196 has 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.

[0076] FIG. 4 is a photograph illustrating the results of the bacterial second-stage test using API (registered trademark) 20E and API (registered trademark) 20NE. As shown in FIG. 4, it was found that the komham196 has activity to metabolize esculin (ESC), lysine hydrochloride (LDC), and gelatin (GEL), and has esterase activity, lysine decarboxylase activity, and gelatinase activity.

[0077] [Table 4]

No.	Enzyme	Substrate	pH	Result	
				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-phosphohydase	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 tetroxide. 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 osmiul. Thereafter, the observation was conducted at a voltage of 5 kV using a SEM (model: JSM-7500F; manufactured by JEOL Ltd.; $\times 20,000$). FIG. 6 shows the results.

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. 7 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 Filtrlong (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-

[0083] FIG. 7 shows the results of the ANI analysis. As shown in FIG. 7, even in the case of the genomic DNA with the highest identity to the base sequence of the genomic DNA of the komham196, the percentage identity was less than 95%. It was confirmed from these results that the komham196 is not a microorganism belonging to the known bacterial species but is a novel species of microorganisms.

[0084] (11) Evaluation of Ability to Utilize Substrate in Compost

An ability of the komham196 to utilize a substrate in compost was examined.

Specifically, compost serving as an isolation source was sterilized twice in an autoclave at 121°C for 20 minutes. The sterilization was conducted to kill microorganisms living in the compost.

Next, the komham196 obtained in Example 1(1) above was inoculated into 80 ml of a PYA liquid culture medium and was cultured for 48 hours. After the culture, centrifugation was conducted at 8000 rpm for 5 minutes. After the centrifugation, the supernatant was removed to harvest the bacteria. After the harvest, washing was conducted twice using sterile water. After the washing, the bacteria were suspended in 3 ml of sterile water. Samples were prepared in 50-ml tubes as shown in Table 5 below, and stationary culture was conducted at 50°C for 48 hours. After the stationary culture, the DNA level in each sample was measured. As a result, proliferation of the bacteria was confirmed in the samples 3 to 6. It was found from these results that the komham196 has an ability to utilize chitin, starch, and casein.

[0085] [Table 5]

	1. NC	2. NC2	3. PC	4. Chitin	5. Starch	6. Casein
Compost	2.0 g	2.0 g	2.0 g	2.0 g	2.0 g	2.0 g
Bacterial suspension	—	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
—PYA (pH 9)	3.5 ml	3.5 ml	3.5 ml	3.5 ml	3.5 ml	3.5 ml
PYA (pH 9)	—	—	0.5 ml	—	—	—
1.0% Chitin	—	—	—	0.5 ml	—	—
1.0% Starch	—	—	—	—	0.5 ml	—
1.0% Casein	—	—	—	—	—	0.5 ml
DW	0.5 ml	—	—	—	—	—

—PYA: culture medium obtained by removing carbon source from composition of PYA culture medium

—: not added

[0086] (12) Proliferation Test

It was found from the results of Example 1(11) above that the komham196 can utilize chitin, starch, or casein, and therefore, a microbial proliferation test was conducted.

Specifically, 250 mg was collected from each of the samples obtained in Example 1(11) above after the stationary culture. After the collection, NucleoSpin (registered trademark) Soil (manufactured by Takara Bio Inc.) was used to extract DNA. After the extraction, the DNA was quantified using Qubit dsDNA BR kit (manufactured by ThermoFisher Scientific Inc.) and Qubit 4 Fluorometer (manufactured by ThermoFisher Scientific Inc.). FIG. 8 shows the results.

[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, 2024, 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

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;
- 5 (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;
- 10 (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.

(Supplementary Note 5)

The microorganism according to Supplementary Note 4, further having a property (9) below:

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

(Supplementary Note 6)

The microorganism according to any one of Supplementary Notes 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.

<Microorganism>

(Supplementary Note 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.

(Supplementary Note 8)

The microorganism according to Supplementary Note 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;

5 (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;

10 (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.

(Supplementary Note 9)

The microorganism according to Supplementary Note 7 or 8, including a genomic DNA having an ANI value of 74% or more relative to a base sequence represented by SEQ ID NO: 2.

15 <Property>

(Supplementary Note 10)

A microorganism

belonging to the family Bacillaceae, and

having properties (1) to (9) below:

20 (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;

25 (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;

30 (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.

(Supplementary Note 11)

The microorganism according to Supplementary Note 10,

belonging to the family Bacillaceae, and

35 including a genomic DNA having an ANI value of 74% or more relative to a base

sequence represented by SEQ ID NO: 2.

<Application>

(Supplementary Note 12)

A composition for use in degradation of garbage, including the microorganism according to any one of Supplementary Notes 1 to 11.

(Supplementary Note 13)

A composition for use in degradation of a polysaccharide and/or a protein, including the microorganism according to any one of Supplementary Notes 1 to 11.

(Supplementary Note 14)

A composition for use in degradation of chitin, starch, and/or casein, including the microorganism according to any one of Supplementary Notes 1 to 11.

<Method>

(Supplementary Note 15)

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

(Supplementary Note 16)

The treatment method according to Supplementary Note 15, wherein the degradation is conducted at a temperature of 30°C to 55°C.

(Supplementary Note 17)

The treatment method according to Supplementary Note 15 or 16, wherein the degradation is conducted at a pH of 9 to 10.

(Supplementary Note 18)

The treatment method according to any one of Supplementary Notes 15 to 17, wherein the garbage is selected from the group consisting of crustacean exoskeletons, fish, meat, and rice.

Industrial Applicability

[0091] As described above, with the present disclosure, it is possible to provide a novel microorganism capable of degrading polysaccharides such as chitin and starch, and/or proteins such as casein, etc. Accordingly, the present invention is very useful in the fields of, for example, waste disposal and the like.

[Sequence Listing]

P23008WO.xml

CLAIMS

1. A microorganism
belonging to the family Bacillaceae, and
5 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,
10 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
15 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,
20 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;
25 (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;
30 (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:
35 (9) having an ability to utilize chitin, starch, and/or casein.

6. The microorganism according to claim 2 or 3, 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.

5

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:
 10 (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.

15

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;
- 20 (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,
 25 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.

30

9. A microorganism
 belonging to the family Bacillaceae, and
 having properties (1) to (9) below:
 (1) being a Gram-positive spore-forming bacillus;
 35 (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,
 - 5 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;
 - 10 (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 3 and 7 to 9.
- 15 11. A garbage treatment method comprising a step of bringing the microorganism according to any one of claims 1 to 3 and 7 to 9 into contact with garbage to allow the microorganism to degrade the garbage.
- 20 12. The treatment method according to claim 11, wherein the degradation is conducted at a temperature of 30°C to 55°C.
13. The treatment method according to claim 11, wherein the degradation is conducted at a pH of 9 to 10.
- 25 14. The treatment method according to claim 11, wherein the garbage is selected from the group consisting of crustacean exoskeletons, fish, meat, and rice.