

DESCRIPTION

**METHOD FOR GENERATING 3,5-DIHYDROXY-4-METHOXYBENZYL ALCOHOL
FROM PLANKTON**

Technical Field

[0001]

The present invention relates to a method for producing 3,5-dihydroxy-4-methoxybenzyl alcohol, the method producing 3,5-dihydroxy-4-methoxybenzyl alcohol serving as an antioxidant from plankton.

Background Art

[0002]

The present inventor has already found a novel antioxidant, 3,5-dihydroxy-4-methoxybenzyl alcohol (hereinafter referred to as "DHMBA"), in heated oyster meat and also succeeded in synthesizing and identifying this substance. Note that DHMBA is not detected in raw oyster meat.

Regarding this matter, it is generally known that, as a biological characteristic of oyster, oyster draws a large quantity of seawater, thereby digestively obtaining plankton as a feed from the large quantity of seawater thus drawn.

Citation List

Patent Literature

[0003]

Patent Literature 1: JP 2016-42825 A

Summary of Invention

Technical Problem

[0004]

Thus, in the present invention, the present inventor built a hypothesis that DHMBA was included in the plankton.

First, DHMBA in the plankton was measured by collecting and filtering the plankton only to find that DHMBA was not detected. Next, when the plankton were collected, filtered, and heated, DHMBA was detected. Further, when the plankton were collected, filtered, and pressurized, DHMBA was detected.

[0005]

Thus, the present inventor contemplated that the aforementioned DHMBA was a useful substance derived from the plankton and therefore invented a method for producing DHMBA from the plankton.

Solution to Problem

[0006]

The present invention includes filtering collected seawater containing plankton using a filter, taking out a cell content from the plankton remained on the filter, and then heating the cell content thus taken out to produce

3,5-dihydroxy-4-methoxybenzyl alcohol from a heated material thus heated, wherein

the plankton is a diatom.

Alternatively, the present invention includes filtering collected seawater containing plankton using a filter, taking out a cell content from the plankton remained on the filter, and then heating the cell content thus taken out to produce 3,5-dihydroxy-4-methoxybenzyl alcohol from a heated material thus heated, wherein

the plankton is a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae or a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae, the order Surirellales, the family Entomoneidaceae, the genus Entomoneis.

Alternatively, the present invention includes filtering collected seawater containing plankton using a filter, crushing the plankton remained on the filter with an addition of an extracting solution, and extracting a cell content from the plankton followed by heating to produce

3,5-dihydroxy-4-methoxybenzyl alcohol from a heated material thus heated, wherein

the plankton is a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class

Bacillariophyceae, the subclass Bacillariophycidae or a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae, the order Surirellales, the family Entomoneidaceae, the genus Entomoneis.

Alternatively, in the present invention, the heating time is at least 1 hour or longer.

Alternatively, the present invention includes filtering collected seawater containing plankton using a filter, taking out a cell content from the plankton remained on the filter, and then pressurizing the cell content thus taken out to produce 3,5-dihydroxy-4-methoxybenzyl alcohol from a pressurized material thus pressurized, wherein

the plankton is a diatom.

Alternatively, the present invention includes filtering collected seawater containing plankton using a filter, taking out a cell content from the plankton remained on the filter, and then pressurizing the cell content thus taken out to produce 3,5-dihydroxy-4-methoxybenzyl alcohol from a pressurized material thus pressurized, wherein

the plankton is a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae or a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass

Bacillariophycidae, the order Surirellales, the family Entomoneidaceae, the genus Entomoneis.

Alternatively, the present invention includes filtering collected seawater containing plankton using a filter, crushing the plankton remained on the filter with an addition of an extracting solution, and extracting a cell content from the plankton followed by pressurization to produce 3,5-dihydroxy-4-methoxybenzyl alcohol from a pressurized material thus pressurized, wherein

the plankton is a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae or a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae, the order Surirellales, the family Entomoneidaceae, the genus Entomoneis.

Alternatively, in the present invention, the pressurization is performed at at least 2 atmospheres or more.

Alternatively, in the present invention, the pressurizing time is at least 1 hour or longer.

Advantageous Effects of Invention

[0007]

The present invention can provide the method for producing the antioxidant DHMBA from the plankton included in the seawater

by collecting the seawater including the plankton, thereby achieving excellent advantageous effects.

Brief Description of Drawings

[0008]

Fig. 1 is an explanatory diagram illustrating filtration of collected seawater.

Fig. 2 is an explanatory diagram illustrating sonication of filtered plankton remained on a filter and subsequent heating extraction of DHMBA.

Fig. 3 is an explanatory diagram illustrating sonication of filtered plankton remained on a filter and subsequent pressurizing extraction of DHMBA.

Fig. 4 is an explanatory diagram (1) illustrating whether or not DHMBA is produced when the plankton present in the seawater collected in a predetermined sea area in Hiroshima, Japan, at a predetermined period are heated.

Fig. 5 is an explanatory diagram (2) illustrating whether or not DHMBA is produced when the plankton present in the seawater collected in the predetermined sea area in Hiroshima, Japan, at the predetermined period are heated.

Fig. 6 is an explanatory diagram illustrating whether or not DHMBA is produced when the plankton present in the seawater collected in a predetermined sea area in Miyagi, Japan, at a predetermined period are heated.

Fig. 7 is an explanatory diagram illustrating whether or not DHMBA is produced when the plankton present in the seawater collected in the predetermined sea area in Miyagi, Japan, at the predetermined period are pressurized.

Fig. 8 is an explanatory diagram (1) illustrating a global search result under a search condition excluding environmental sequences.

Fig. 9 is an explanatory diagram (2) illustrating the global search result under the search condition excluding the environmental sequences.

Fig. 10 is an explanatory diagram (1) illustrating the search result under the search condition in which the search is limited to sequences from type material.

Fig. 11 is an explanatory diagram (2) illustrating the search result under the search condition in which the search is limited to the sequences from type material.

Fig. 12 is an explanatory diagram (3) illustrating the global search result under the search condition excluding the environmental sequences.

Fig. 13 is an explanatory diagram (4) illustrating the global search result under the search condition excluding the environmental sequences.

Fig. 14 is an explanatory diagram (5) illustrating the global search result under the search condition excluding the environmental sequences.

Fig. 15 is an explanatory diagram (3) illustrating the search result under the search condition in which the search is limited to the sequences from type material.

Fig. 16 is an explanatory diagram (4) illustrating the search result under the search condition in which the search is limited to the sequences from type material.

Fig. 17 is an explanatory diagram (5) illustrating the search result under the search condition in which the search is limited to the sequences from type material.

Fig. 18 is an explanatory diagram (6) illustrating the global search result under the search condition excluding the environmental sequences.

Fig. 19 is an explanatory diagram (7) illustrating the global search result under the search condition excluding the environmental sequences.

Fig. 20 is an explanatory diagram (8) illustrating the global search result under the search condition excluding the environmental sequences.

Fig. 21 is an explanatory diagram (6) illustrating the search result under the search condition in which the search is limited to the sequences from type material.

Fig. 22 is an explanatory diagram (7) illustrating the search result under the search condition in which the search is limited to the sequences from type material.

Fig. 23 is an explanatory diagram (8) illustrating the

search result under the search condition in which the search is limited to the sequences from type material.

Fig. 24 is an explanatory diagram (9) illustrating the global search result under the search condition excluding the environmental sequences.

Fig. 25 is an explanatory diagram (10) illustrating the global search result under the search condition excluding the environmental sequences.

Fig. 26 is an explanatory diagram (11) illustrating the global search result under the search condition excluding the environmental sequences.

Fig. 27 is an explanatory diagram (9) illustrating the search result under the search condition in which the search is limited to the sequences from type material.

Fig. 28 is an explanatory diagram (10) illustrating the search result under the search condition in which the search is limited to the sequences from type material.

Fig. 29 is an explanatory diagram (11) illustrating the search result under the search condition in which the search is limited to the sequences from type material.

Description of Embodiments

[0009]

First, the seawater was collected in a sea area where oyster farming was primarily performed (e.g., sea areas in

Hiroshima and Miyagi, Japan) at a predetermined period and the collected seawater was filtered to take out plankton 1 remained on a filter used for filtration. The plankton 1 were heated or pressurized and then subjected to examination and analysis to determine whether or not DHMBA was detected.

[0010]

First, detection of DHMBA after heating will be described.

The seawater was collected in an actual sea area where oyster farming was performed (e.g., sea areas in Hiroshima and Miyagi, Japan).

[0011]

(Collection of seawater including plankton)

The seawater is pumped up by a pump or the like in the sea area where oyster farming is performed (e.g., the sea areas in Hiroshima and Miyagi, Japan) at a predetermined period. Specifically, the seawater including the plankton is pumped up in the sea area in Hiroshima in September and March and in the sea area in Miyagi in May.

Note that, in the present example, the seawater in the sea areas in Hiroshima and Miyagi was collected. However, the invention is not limited to the seawater in Hiroshima and Miyagi.

Further, regarding the period, the invention is not limited to the seawater in September or March.

[0012]

(Filtration of seawater including plankton)

The seawater including the plankton 1 was collected and then filtrated through a filter formed of a nonwoven fabric or the like, for example, a GF/C filter 4 (see Fig. 1).

In this operation, the amount of the seawater to be filtered was not limited. This time, about 3100 liters of seawater was collected in each sea area in Hiroshima and Miyagi and filtered through the filter.

Note that the filter used for filtration in the above method has a large amount of the plankton 1 adhered thereon, and the filter on which the plankton 1 are adhered can be frozen and stored until an extraction operation is performed.

[0013]

(Ultrasonic treatment)

Next, for example, in a case where the filter on which the plankton 1 are adhered is frozen and stored, the filter which is frozen and stored is put into a container 5 such as a centrifuge tube, the container 5 is filled with, for example, ultrapure water, and the plankton 1 are further subjected to, for example, sonication using ultrasonic waves for about 1 hour or a ball mill treatment to destroy cell walls, thereby facilitating extraction of DHMBA and the like.

[0014]

(Extraction)

After the plankton 1 cells are destroyed by the aforementioned method such as sonication, the cells thus

destroyed are heated to take out contents of the cells. That is, the aforementioned plankton and the like are subjected to hot water bath extraction at about 100°C or the like (see Fig. 2).

In this operation, the plankton 1 in the aforementioned container 5 is heated in the hot water bath extraction and is not directly heated. That is, the container 5 is put into the hot water which is heated and stored in a beaker or the like to perform so-called indirect heating. This is advantageous, for example, in that stable heating extraction can be performed with a constant heating temperature.

[0015]

(Sampling)

Then, samples were taken every hour for five hours during the aforementioned hot water bath extraction.

[0016]

(Analysis of useful substance)

The concentration of DHMBA in the plankton 1 taken as the aforementioned sample was measured by LC-MS/MS.

[0017]

(Result of extraction experiment with samples in Hiroshima sea area)

As a result, DHMBA was not detected in the plankton before heating in both the aforementioned samples taken in the Hiroshima sea area in September 2016 and March 2017.

However, in the sample taken in the Hiroshima sea area in September, it could be confirmed that DHMBA was detected with an extraction amount of 0.303 (ng/L) after heating, that is, after the hot water bath extraction for 1 hour. Further, it could be confirmed that DHMBA was detected with an extraction amount of 0.297 (ng/L) after the hot water bath extraction for 2 hours and DHMBA was detected with an extraction amount of 0.279 (ng/L) after the hot water bath extraction for 3 hours. Further, it could be confirmed that DHMBA was detected with an extraction amount of 0.274 (ng/L) after the hot water bath extraction for 4 hours and DHMBA was detected with an extraction amount of 0.217 (ng/L) after the hot water bath extraction for 5 hours.

[0018]

Further, in the sample taken in the Hiroshima sea area in March, it could be confirmed that DHMBA was detected with an extraction amount of 0.906 (ng/L) after heating, that is, after the hot water bath extraction for 1 hour. Further, it could be confirmed that DHMBA was detected with an extraction amount of 0.66 (ng/L) after the hot water bath extraction for 2 hours and DHMBA was detected with an extraction amount of 0.686 (ng/L) after the hot water bath extraction for 3 hours. Further, it could be confirmed that DHMBA was detected with an extraction amount of 0.517 (ng/L) after the hot water bath extraction for 4 hours and DHMBA was detected with an extraction amount of 0.794 (ng/L) after the hot water bath extraction for 5 hours (see Fig.

4 and Fig. 5).

[0019]

(Result of extraction experiment with samples in Miyagi sea area)

Fig. 6 shows a detection result of DHMBA with samples taken in the Miyagi sea area in May 2016.

[0020]

Fig. 6 shows changes in the extraction amount of DHMBA over time. Same as the sample in the Hiroshima sea area, DHMBA was not detected in the plankton before heating in the sample before heating. However, it could be confirmed that DHMBA was detected with an extraction amount of 0.108 (ng/L) after heating, that is, after the hot water bath extraction for 1 hour. Further, it could be confirmed that DHMBA was detected with an extraction amount of 0.113 (ng/L) after the hot water bath extraction for 2 hours and DHMBA was detected with an extraction amount of 0.156 (ng/L) after the hot water bath extraction for 3 hours. Further, it could be confirmed that DHMBA was detected with an extraction amount of 0.125 (ng/L) after the hot water bath extraction for 4 hours and DHMBA was detected with an extraction amount of 0.134 (ng/L) after the hot water bath extraction for 5 hours (see Fig. 6).

[0021]

Note that the heating time is not limited to one-hour time lapse interval. DHMBA may be detected with a heating time of less than one hour.

In any case, it was found that DHMBA was not detected in the plankton which were not heated, while DHMBA could be quickly detected in the plankton which were heated.

[0022]

Next, detection of DHMBA after pressurization will be described.

First, the seawater was collected in an actual sea area where oyster farming was performed (e.g., a sea area in Miyagi, Japan).

[0023]

(Collection of seawater including plankton)

The seawater is pumped up by a pump or the like in the sea area where oyster farming is performed (e.g., the sea area in Miyagi, Japan) at a predetermined period. Specifically, the seawater including the plankton is pumped up in the sea area in Miyagi in May 2016.

Note that, in the present example, the seawater in the sea areas in Miyagi was collected. However, the invention is not limited to the seawater in Miyagi. Further, regarding the period, the invention is not limited to the seawater in May.

[0024]

(Filtration of seawater including plankton)

The seawater including the plankton 1 was collected and then filtrated through a filter formed of a nonwoven fabric or the like, for example, a GF/C filter 4 (see Fig. 1).

In this operation, the amount of the seawater to be filtered was not limited. This time, about 3100 liters of seawater was collected in the sea area in Miyagi and filtered through the filter.

Note that the filter used for filtration in the above method has a large amount of the plankton 1 adhered thereon, and the filter on which the plankton 1 are adhered can be frozen and stored until an extraction operation is performed.

[0025]

(Ultrasonic treatment)

Next, for example, in a case where the filter on which the plankton 1 are adhered is frozen and stored, the filter which is frozen and stored is put into a container 5 such as a centrifuge tube, the container 5 is filled with, for example, ultrapure water, and the plankton 1 are further subjected to, for example, sonication using ultrasonic waves for about 1 hour or a ball mill treatment to destroy cell walls, thereby facilitating extraction of DHMBA and the like.

[0026]

(Extraction)

Subsequently, after the plankton 1 cells are destroyed by the aforementioned method such as sonication, the cells thus destroyed are pressurized to take out contents of the cells. That is, the aforementioned plankton and the like were pressurized to about 2 atmospheres to perform extraction (see

Fig. 3).

In this operation, the pressurizing method is not limited. As a general method, a pressure pot may be used for performing pressurization. Further, other methods may be used for performing pressurization.

[0027]

(Sampling)

Then, samples were taken every hour for five hours during the aforementioned pressurizing extraction.

[0028]

(Analysis of useful substance)

The concentration of DHMBA in the plankton 1 taken as the aforementioned sample was measured by LC-MS/MS.

[0029]

(Result of extraction experiment with samples in Miyagi sea area)

Fig. 7 shows a detection result of DHMBA with samples taken in the Miyagi sea area.

[0030]

Fig. 7 shows changes in the extraction amount of DHMBA over time. DHMBA was not detected in the plankton before pressurization. It could be confirmed that DHMBA was detected with an extraction amount of 0.05 (ng/L) after pressurization at 2 atmospheres, that is, after pressurization at 2 atmospheres for 1 hour. Further, it could be confirmed that DHMBA was detected with an extraction amount of 0.29 (ng/L) after

pressurization at 2 atmospheres for 2 hours and DHMBA was detected with an extraction amount of 0.49 (ng/L) after pressurization at 2 atmospheres for 3 hours. Further, it could be confirmed that DHMBA was detected with an extraction amount of 0.58 (ng/L) after pressurization at 2 atmospheres for 4 hours and DHMBA was detected with an extraction amount of 0.67 (ng/L) after pressurization at 2 atmospheres for 5 hours (see Fig. 7).

[0031]

In any case, it was found that DHMBA was not detected in the plankton which were not pressurized, while DHMBA could be quickly detected in the plankton which were pressurized.

[0032]

(Identification of plankton 1 in which DHMBA can be detected)

There are many kinds of the plankton 1 in the seawater and it was unclear in which kinds of the plankton 1 DHMBA was detected. Thus, the present inventor decided to identify the plankton 1 in which DHMBA was detected.

The present inventor collected the seawater as described above to obtain the plankton 1 included in the seawater.

Among many kinds of the plankton 1 thus obtained, about 200 kinds of the plankton 1 were selected and cultured separately.

Then, about 200 kinds of the plankton thus cultured were subjected to the aforementioned DHMBA extraction treatment. That is, the plankton were subjected to the aforementioned ultrasonic treatment, the aforementioned extraction treatment

with heating or pressurization, and the like.

[0033]

As a result, DHMBA was detected in 4 microalgal strains in the plankton¹. Further, detailed morphological observation of these 4 microalgal strains could identify them as diatoms.

Note that diatoms are most ubiquitous in phytoplankton and diverse in many kinds.

However, when the present inventor observed the morphology of the aforementioned 4 microalgal strains in which DHMBA was detected in detail using a microscope, all 4 strains turned out to be diatoms. The microscope used in this observation has 1,000-fold magnification (ocular lens: 10-fold, objective lens: 100-fold).

The aforementioned 4 microalgal strains, that is, the diatoms, are members of marine diatoms widespread in the seawater in bays and known to abundantly produce unsaturated fatty acids. Further, they are also known as super-planktonic diatoms.

[0034]

Next, the present inventor planned to perform DNA analysis of the aforementioned 4 microalgal strains, that is, the diatoms, for further specific identification of the diatoms.

The DNA analysis is performed to determine the taxonomic rank that the diatoms belong to. The aforementioned taxonomic rank is classified into "phylum", "subphylum", "class", "subclass", "order", "family", "genus", and "species", and the

classification levels become more specific in the order from “phylum” to “species”

Processes of the DNA analysis will be described below.

[0035]

Classification of diatom (H-7-09 strain) in which DHMBA is detected

It is speculated that the diatom belongs to the subclass Bacillariophycidae. However, classification was not made at the level of “order” or below.

[0036]

(Method)

Performing megaBLAST search with GenBank (NCBI, NHI)

Search condition (1) global search excluding environmental sequences (Fig. 8, Fig. 9)

-> Discussion will be provided based on these results.

Search condition (2) search limited to sequences from type material (Fig. 10, Fig. 11)

-> Discussion will not be provided based on these results due to insufficient information amount of sequence data for comparison.

Site	Sequence name	Base sequence length used for analysis	Sequence assembly	Blast analysis result
18S	H7-09-18S	1223 bp	Performed	Fig. 8
28S	H7-09-28S-D2R2	474 bp	Not performed	Fig. 9

[0037]

(megaBLAST search result)

· 18S

Showing about 96.2% homology with the sequence of the *Humidophila schmassmannii* isolate HYU-D030 strain.

-> Homology is as low as about 96%.

However, considering sequences found in the second and following places, it is speculated that the diatom belongs to the class Bacillariophyceae, the subclass Bacillariophycidae.

· 28S D2R2 sequence

Showing about 96.1% homology with the sequence of the *Pseudo-nitzschia multistriata* HAB-132 strain.

-> Homology is as low as about 96%. Further, short sequence and low score and coverage make the classification result less reliable as compared with the result of 18S.

However, the result is not contradictory to the possibility that the diatom belongs to the subclass Bacillariophycidae as speculated from the result of 18S.

[0038]

(Classification result)

It is speculated that the diatom belongs to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae.

However, classification cannot be made at the level of "order" or below.

[0039]

Classification of diatom (H-9-05 strain) in which DHMBA is detected

It is speculated that the diatom belongs to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae, the order Surirellales, the family Entomoneidaceae, the genus Entomoneis.

However, classification was not made at the level of "species".

[0040]

(Method)

Performing megaBLAST search with GenBank (NCBI, NHI)

Search condition (1) global search excluding the environmental sequences (Fig. 12, Fig. 13, Fig. 14)

-> Discussion will be provided based on these results.

Search condition (2) search limited to the sequences from type material (Fig. 15, Fig. 16, Fig. 17)

-> Discussion will not be provided based on these results due to insufficient information amount of sequence data for comparison.

Site	Sequence name	Base sequence length used for analysis	Sequence assembly	Blast analysis result
18S	H9-05-18S	1204 bp	Performed	Fig. 12
28S	H9-05-28S-D2C2	480 bp	Not performed	Fig. 13
28S	H9-05-28S-D2R2	458 bp	Not performed	Fig. 14

[0041]

(megaBLAST search result)

- 18S

Showing 98% homology with the sequence of the *Entomoneis paludosa* L431 strain.

-> Homology is as low as 98%. However, considering that sequences of the genus *Entomoneis* are found in the second and following places, it is speculated that the diatom belongs to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae, the order Surirellales, the family Entomoneidaceae, the genus *Entomoneis*.

- 28S D2C2 sequence

Showing about 92% homology with the sequence of the *Entomoneis ornata* 27D strain.

-> It has a very low homology of 92%, providing less useful information. Further, short sequence and low score and coverage make the classification result significantly less reliable as compared with the result of 18S.

However, the result is not contradictory to the possibility that the diatom belongs to the genus *Entomoneis* as speculated from the result of 18S.

- 28S D2R2 sequence

Showing about 96% homology with the sequence of the *Pseudo-nitzschia multistriata* HAB-132 strain.

-> It has a low homology of 96%, providing less useful information. Further, short sequence and low score and coverage as compared with D2C2 make the classification result significantly less reliable.

However, the result is not contradictory to the possibility that the diatom belongs to the subphylum Bacillariophytina as speculated from the result of 18S.

[0042]

(Classification result)

It is speculated that the diatom belongs to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae, the order Surirellales, the family Entomoneidaceae, the genus Entomoneis.

However, classification was not made at the level of "species".

[0043]

Classification of diatom (H-9-06 strain) in which DHMBA is detected

It is speculated that the diatom belongs to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae, the order Surirellales, the family Entomoneidaceae, the genus Entomoneis.

However, classification was not made at the level of "species".

[0044]

(Method) Performing megaBLAST search with GenBank (NCBI, NHI)

Search condition (1) global search excluding the environmental sequences (Fig. 18, Fig. 19, Fig. 20)

-> Discussion will be provided based on these results.

Search condition (2) search limited to the sequences from type material (Fig. 21, Fig. 22, Fig. 23)

-> Discussion will not be provided based on these results due to insufficient information amount of sequence data for comparison.

Site	Sequence name	Base sequence length used for analysis	Sequence assembly	Blast analysis result
18S	H9-06-18S	1204bp	Performed	Fig. 18
28S	H9-06-28S-D2C2	440 bp	Not performed	Fig. 19
28S	H9-06-28S-D2R2	609 bp	Not performed	Fig. 20

[0045]

(megaBLAST search result)

· 18S

Showing 99% homology with the sequence of the *Entomoneis paludosa* L431 strain.

-> Considering that homology is 99% and sequences of the genus *Entomoneis* are found in the second and following places, it is speculated that the diatom belongs to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae, the order

Surirellales, the family Entomoneidaceae, the genus Entomoneis.

- 28S D2C2 sequence

Showing 91% homology with the sequence of the Entomoneis ornata 27D strain.

-> It has a very low homology of 91%, providing less useful information. Further, short sequence and low score make the classification result significantly less reliable as compared with the result of 18S.

However, the result is not contradictory to the possibility that the diatom belongs to the genus Entomoneis as speculated from the result of 18S.

- 28S D2R2 sequence

Showing 88% homology with Vannella septentrionalis (ameba).

Further, it had 79% or less homology with a fungus forming an arbuscular mycorrhiza.

-> Homology is less than 90%. Further, short sequence and lower score and coverage as compared with D2C2 make the classification result significantly less reliable.

Thus, the result is excluded from the present discussion on classification.

[0046]

(Classification result)

It is speculated that the diatom belongs to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class

Bacillariophyceae, the subclass Bacillariophycidae, the order Surirellales, the family Entomoneidaceae, the genus Entomoneis.

[0047]

H-9-09 strain Classification

Undetermined

[0048]

(Method) Performing megaBLAST search with GenBank (NCBI, NHI)

Search condition (1) global search excluding the environmental sequences (Fig. 24, Fig. 25, Fig. 26)

-> Discussion will be provided based on these results.

Search condition (2) search limited to the sequences from type material (Fig. 27, Fig. 28, Fig. 29)

-> Discussion will not be provided based on these results due to insufficient information amount of sequence data for comparison.

Site	Sequence name	Base sequence length used for analysis	Sequence assembly	Blast analysis result
18S	H9-09-18S	1228 bp	Performed	Fig. 24
28S	H9-09-28S-D2C2	511 bp	Not performed	Fig. 25
28S	H9-09-28S-D2R2	441 bp	Not performed	Fig. 26

[0049]

(megaBLAST search result)

· 18S

Showing 97% homology with the sequence of the *Caecitellus*

paraparvulus HFCC320 strain (*Bicosoeca*).

Sequences of diatoms of the same genus are found in the second and following places with a homology of 97%.

-> It has a low homology of 97%. However, this cannot rule out the possibility that the sequence of a *Bicosoeca* is amplified.

However, prior microscopic observation confirmed that the specimen had a diatom-like morphology.

- 28S D2C2 sequence

Showing 88% homology with the sequence of the *Caecitellus* paraparvulus HFCC71 strain (*Bicosoeca*).

-> Homology is less than 90%. Further, short sequence and lower score and coverage make the classification result significantly less reliable as compared with the result of 18S.

Thus, the result is excluded from the present discussion on classification.

However, the result is not contradictory to the result of 18S (indicating a *Bicosoeca*).

- 28S D2R2 sequence

Showing about 92% homology with the sequence of the *Pseudo-nitzschia multistriata* HAB-132 strain (subphylum Bacillariophytina).

-> It has a low homology of 92%. Further, short sequence and low score and coverage make the classification result less reliable as compared with the result of 18S.

However, the result is not contradictory to the prior morphological observation (indicating a diatom).

[0050]

(Classification result)

Undetermined

Reference Signs List

[0051]

1 Plankton

4 GF/C filter

5 Container

CLAIMS

1. A method for producing 3,5-dihydroxy-4-methoxybenzyl alcohol from plankton, comprising:

filtering collected seawater containing the plankton using a filter;

taking out a cell content from the plankton remained on the filter; and then

heating the cell content thus taken out to produce 3,5-dihydroxy-4-methoxybenzyl alcohol from a heated material thus heated, wherein

the plankton is a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae or a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae, the order Surirellales, the family Entomoneidaceae, the genus Entomoneis.

2. A method for producing 3,5-dihydroxy-4-methoxybenzyl alcohol from plankton, comprising:

filtering collected seawater containing the plankton using a filter;

crushing the plankton remained on the filter with an addition of an extracting solution, and

extracting a cell content from the plankton followed

by heating to produce 3,5-dihydroxy-4-methoxybenzyl alcohol from a heated material thus heated, wherein

the plankton is a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae or a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae, the order Surirellales, the family Entomoneidaceae, the genus Entomoneis.

3. The method for producing 3,5-dihydroxy-4-methoxybenzyl alcohol from the plankton according to claim 1, wherein the heating time is at least 1 hour or longer.

4. A method for producing 3,5-dihydroxy-4-methoxybenzyl alcohol from plankton, comprising:

filtering collected seawater containing the plankton using a filter;

taking out a cell content from the plankton remained on the filter, and then

pressurizing the cell content thus taken out to produce 3,5-dihydroxy-4-methoxybenzyl alcohol from a pressurized material thus pressurized, wherein

the plankton is a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class

Bacillariophyceae, the subclass Bacillariophycidae or a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae, the order Surirellales, the family Entomoneidaceae, the genus Entomoneis.

5. A method for producing 3,5-dihydroxy-4-methoxybenzyl alcohol from plankton, comprising:

filtering collected seawater containing the plankton using a filter;

crushing the plankton remained on the filter with an addition of an extracting solution, and

extracting a cell content from the plankton followed by pressurization to produce 3,5-dihydroxy-4-methoxybenzyl alcohol from a pressurized material thus pressurized, wherein

the plankton is a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae or a diatom belonging to the phylum Bacillariophyta, the subphylum Bacillariophytina, the class Bacillariophyceae, the subclass Bacillariophycidae, the order Surirellales, the family Entomoneidaceae, the genus Entomoneis.

6. The method for producing 3,5-dihydroxy-4-methoxybenzyl

alcohol from the plankton according to claim 4 or 5,
wherein the pressurization is performed at at least 2
atmospheres or more.

7. The method for producing 3,5-dihydroxy-4-methoxybenzyl
alcohol from the plankton according to claim 4 or 5,
wherein the pressurizing time is at least 1 hour or longer.