Non-patent document 9: Matsumoto, M., et al., "Impact of intestinal microbiota on intestinal luminal metabolome." Scientific Reports 2012; 2: 233.
Non-patent document 10: Dietmair, S., et al., "Towards quantitative metabolomics of mammalian cells: development of a metabolite extraction protocol." Analytical Biochemistry; 404 (2), 2010, pp. 155-164.
Non-patent document 11: Kuwabara, H., et al., "Altered metabolites in the plasma of autism spectrum disorder: a capillary electrophoresis time-of-flight mass spectroscopy study." PLoS One 2013; 8 (9): e73814.
Non-patent document 12: Wang, X., et al., "Evaluation and optimization of sample preparation methods for metabolic profiling analysis of Escherichia coli." Electrophoresis 2015.

Summary

Object to be Solved by the Disclosure

[0008]

An object of the present disclosure is to provide a more practical method for extracting substances from a fecal sample. More specifically, an object of the present disclosure is to provide a method for extracting substances from a fecal sample, the method being suitable for conducting analyses such as metabolomic analysis using mass spectrometry or nuclear magnetic resonance spectrometry, and metagenomic analysis using a next-generation sequencer.

Means to Solve the Object

[0009]

The present inventors have conducted diligent studies to attain the objects and consequently completed the present disclosure by finding that the objects can be attained by a method for extracting one or more substances from a fecal sample, comprising:

(A) step A of suspending the fecal sample in any one of the following solvents (a) to (g) to obtain a suspension:

(a) a solvent comprising an aqueous solvent and a hydrophilic organic solvent;

(b) a solvent comprising an aqueous solvent and a hydrophobic organic solvent;

(c) a solvent comprising an aqueous solvent, a hydrophilic organic solvent and a hydrophobic organic solvent;

(d) a solvent comprising a hydrophilic organic solvent;

(e) a solvent comprising a hydrophobic organic solvent;

(f) a solvent comprising a hydrophilic organic solvent and a hydrophobic organic solvent; and

(g) a solvent consisting of water;

(B) step B of separating a liquid layer comprising the solvent from the suspension; and

(C) step C of removing proteins from the liquid layer and then obtaining the one or more substances.

[0009a]

In one aspect, the disclosure refers to a method for extracting one or more substances from a fecal sample, comprising:

step A of suspending the fecal sample in any one of the following solvents (a) to (f) to obtain a suspension:

(a) a solvent comprising an aqueous solvent and a hydrophilic organic solvent;

(b) a solvent comprising an aqueous solvent, a hydrophilic organic solvent and a hydrophobic organic solvent;

(c) a solvent comprising a hydrophilic organic solvent;

(d) a solvent comprising a hydrophobic organic solvent;

(e) a solvent comprising a hydrophilic organic solvent and a hydrophobic organic solvent; and

(f) a solvent consisting of water;

step B of separating a liquid layer comprising the solvent from the suspension; and

step C of removing proteins from the liquid layer and then obtaining the one or more substances,

wherein the step A is step A-1 of subjecting the fecal sample to disruption and suspending treatment with beads having a diameter in a range of 0.02 to 0.5 mm, in any one of the solvents (a) to (f) to obtain a suspension, and/or

the method further comprises before the step A or the step A-1, step X of subjecting a dried fecal sample to disruption treatment with beads having a diameter in a range of 0.6 to 15 mm, to obtain dried fecal small pieces,

wherein the dried fecal small pieces are used as the fecal sample of the step A or the step A-1.

[0009b]

In another aspect, the disclosure refers to a method for identifying one or more substances in a fecal sample, and measuring a concentration, composition or proportion of the one or more substances after performing a method for extracting the one or more substances in the fecal sample, wherein the method for extracting the one or more substances in the fecal sample is the method as described herein, and the method for identifying one or more substances in a fecal sample, and measuring a concentration, composition or proportion of the one or more substances, comprises: step P of separating the one or more substances obtained in step C of the method for extracting by chromatography or capillary electrophoresis, and conducting mass spectrometry and/or nuclear magnetic resonance spectrometry to identify the one or more substances, and to measure the concentration, composition or proportion of the one or more substances.

[0009c]

In still another aspect, the disclosure refers to a method for classifying a plurality of subjects into two or more clusters, comprising:

step Q of conducting the method according to the method as described herein on each of fecal samples obtained from the plurality of subjects to identify two or more substances and to measure a concentration, composition or proportion of the two or more substances; and

step S of conducting cluster analysis on data of the concentration, composition or proportion of the two or more substances obtained in the step Q, and classifying the plurality of subjects into two or more clusters according to similarity of substance profiles.

[0010]

The present inventors have also found that more types of substances can be extracted by combined use of an aqueous solvent with a hydrophilic organic solvent and/or a hydrophobic organic solvent. The present inventors have further found that metabolites such as amino acids can be more efficiently extracted by disruption and suspending treatment using beads in suspending the fecal sample in the solvent. Furthermore, the present inventors have separated the substances extracted from the fecal sample by capillary electrophoresis or chromatography, conducted mass spectrometry and/or nuclear magnetic resonance spectrometry to identify the one or more substances and to measure the concentrations of the one or more substances, and conducted cluster analysis on the measurement data. As a result, the present inventors have found that each characteristic substance in substance profiles can be classified into a cluster (group). The present inventors have further gained these and other findings and completed the present disclosure.

[0011]

Furthermore, the present inventors have compared water and a PBS solution used as aqueous solvents and consequently found that more types of metabolites can be detected by using water. In the case of using a PBS

solution, the present inventors have also found that when a sample is enriched and subjected to mass spectrometry, current flows excessively due to the influence of a salt concentration so that accurate measurement cannot be performed. The present inventors have further gained these and other findings and completed the present disclosure.

[0012]

Moreover, in the case of preserving a fecal sample without being frozen after harvesting of the fecal sample and before extraction of substances from the fecal sample, the present inventors have found that, when the fecal sample is preserved in any one of solvents (h) and (i) given below, variations in metabolite profiles in the fecal sample during the preservation are suppressed. On the basis of these findings, the present disclosure has been completed.

(h) A solvent comprising an aqueous solvent and a hydrophilic organic solvent having a high concentration (e.g., 75% by weight or higher); and

(i) a solvent comprising an aqueous solvent, a hydrophilic organic solvent and a hydrophobic organic solvent.

[0013]

Also described in the present disclosure is:

(1) a method for extracting one or more substances from a fecal sample, comprising:

step A of suspending the fecal sample in any one of the following solvents (a) to (g) to obtain a suspension:

(a) a solvent comprising an aqueous solvent and a hydrophilic organic solvent;

(b) a solvent comprising an aqueous solvent and a hydrophobic organic solvent;

(c) a solvent comprising an aqueous solvent, a hydrophilic organic solvent and a hydrophobic organic solvent;

(d) a solvent comprising a hydrophilic organic solvent;

(e) a solvent comprising a hydrophobic organic solvent;

(f) a solvent comprising a hydrophilic organic solvent and a hydrophobic organic solvent; and

(g) a solvent consisting of water;

step B of separating a liquid layer comprising the solvent from the suspension; and

step C of removing proteins from the liquid layer and then obtaining the one or more substances;

(2) the method for extracting one or more substances according to (1), wherein the step A is step A-1 of subjecting the fecal sample to disruption and suspending treatment with beads in any one of the solvents (a) to (g) to obtain a suspension;

(3) the method for extracting one or more substances according to (1) or (2), wherein the solvent comprises an aqueous solvent and a hydrophilic organic solvent, and a content of water in the solvent is in a range of 10 to 50% by weight;

(4) the method for extracting one or more substances according to (2) or (3), wherein a diameter of the beads in the step A-1 is in a range of 0.02 to 0.5 mm;

(5) the method for extracting one or more substances according to any one of (1) to (4), further comprising, before the step A or the step A-1, step X of subjecting a dried fecal sample to disruption treatment with beads to obtain dried fecal small pieces, wherein the dried fecal small pieces are used as the fecal sample of the step A or the step A-1;

(6) the method for extracting one or more substances according to (5), wherein a diameter of the beads in the step X is in a range of 0.6 to 15 mm;

(7) the method for extracting one or more substances according to any one of (3) to (6), wherein the solvent further comprises a hydrophobic organic solvent;

(8) the method for extracting one or more substances according to any one of (1) to (4) and (7), further comprising, before the step A or the step A-1, step Y of preserving a harvested fecal sample in the following solvent (h) or (i), wherein the fecal sample thus preserved in the step Y is used as the fecal sample of the step A or the step A-1:

(h) a solvent comprising an aqueous solvent and 75% by weight or more of a hydrophilic organic solvent; and

(i) a solvent comprising an aqueous solvent, a hydrophilic organic solvent and a hydrophobic organic solvent;

(9) the method for extracting one or more substances according to any one of (1) to (8), wherein the aqueous

solvent is selected from the group consisting of water, an aqueous solution of salt, and an aqueous buffer solution;

(10) the method for extracting one or more substances according to any one of (1) to (9), wherein the hydrophilic organic solvent is one or more solvents selected from the group consisting of methanol, ethanol, propanol, isopropanol, butanol, isobutanol, acetonitrile, dimethyl sulfoxide, dimethylformamide, dimethylacetamide, acetone, tetrahydrofuran and diethylene glycol; and

(11) the method for extracting one or more substances according to any one of (1) to (10), wherein the hydrophobic organic solvent is one or more solvents selected from the group consisting of chloroform, hexane, ether, diethyl ether, benzene, phenol, and isoamyl alcohol.

[0014]

The present disclosure also relates to:

(12) a method for identifying one or more substances in a fecal sample, and measuring a concentration, composition or proportion of the one or more substances, comprising:

step P of separating the one or more substances extracted by the method according to any one of (1) to (11) by chromatography or capillary electrophoresis, and conducting mass spectrometry and/or nuclear magnetic resonance spectrometry to identify the one or more substances and to measure the concentration, composition or proportion of the one or more substances;

(13) a method for classifying a plurality of subjects into two or more clusters, comprising:

step Q of conducting the method according to (12) on each of fecal samples obtained from the plurality of subjects to identify two or more substances and to measure a concentration, composition or proportion of the two or more substances; and

step S of conducting cluster analysis on data of the concentration, composition or proportion of the two or more substances obtained in the step Q, and classifying the plurality of subjects into two or more clusters according to similarity of substance profiles; and

(14) the method according to (13), further comprising, between the step Q and the step S,

step R of standardizing the data of the concentration, composition or proportion of the two or more substances obtained in the step Q, wherein

the data standardized in the step R is used in the cluster analysis in the step S.

Effect of the Disclosure

[0015]

According to the present disclosure, one or more substances (preferably, intestinal metabolites) can be efficiently extracted from a fecal sample. According to the present disclosure, more types of substances can be extracted from a fecal sample. Accordingly, the present disclosure can provide a method for extracting substances from a fecal sample, the method being suitable for conducting analyses such as metabolomic analysis using mass spectrometry or nuclear magnetic resonance

spectrometry, and metagenomic analysis using a next-generation sequencer.

Brief Description of Drawings

[0016]

[Figure 1] Figure 1 is a diagram showing a flow chart of a method for extracting metabolites.

[Figure 2] Figure 2 is a diagram showing profiles of (cationic) metabolites detected in extraction samples 1 to 5. The hierarchical clustering of samples and metabolites was conducted using Pearson correlation coefficient. In

CLAIMS

1. A method for extracting one or more substances from a fecal sample, comprising:

step A of suspending the fecal sample in any one of the following solvents (a) to (f) to obtain a suspension:

(a) a solvent comprising an aqueous solvent and a hydrophilic organic solvent;

(b) a solvent comprising an aqueous solvent, a hydrophilic organic solvent and a hydrophobic organic solvent;

(c) a solvent comprising a hydrophilic organic solvent;

(d) a solvent comprising a hydrophobic organic solvent;

(e) a solvent comprising a hydrophilic organic solvent and a hydrophobic organic solvent; and

(f) a solvent consisting of water;

step B of separating a liquid layer comprising the solvent from the suspension; and

step C of removing proteins from the liquid layer and then obtaining the one or more substances,

wherein the step A is step A-1 of subjecting the fecal sample to disruption and suspending treatment with beads having a diameter in a range of 0.02 to 0.5 mm, in any one of the solvents (a) to (f) to obtain a suspension, and/or

the method further comprises before the step A or the step A-1, step X of subjecting a dried fecal sample to disruption treatment with beads having a diameter in a

range of 0.6 to 15 mm, to obtain dried fecal small pieces, wherein the dried fecal small pieces are used as the fecal sample of the step A or the step A-1.

2. The method for extracting one or more substances according to claim 1, wherein both the step A-1 and the step X are performed.

3. The method for extracting one or more substances according to claim 1 or 2, wherein the solvent comprises an aqueous solvent and a hydrophilic organic solvent, and a content of water in the solvent is in a range of 10 to 50% by weight.

4. The method for extracting one or more substances according to any one of claims 1 to 3, wherein the solvent further comprises a hydrophobic organic solvent.

5. The method for extracting one or more substances according to any one of claims 1 to 4, further comprising, before the step A or the step A-1, step Y of preserving a harvested fecal sample in the following solvent (h) or (i), wherein the fecal sample thus preserved in the step Y is used as the fecal sample of the step A or the step A-1:

(h) a solvent comprising an aqueous solvent and 75% by weight or more of a hydrophilic organic solvent; and

(i) a solvent comprising an aqueous solvent, a hydrophilic organic solvent and a hydrophobic organic solvent.

6. The method for extracting one or more substances according to any one of claims 1 to 5, wherein the aqueous solvent is selected from the group consisting of water, an aqueous solution of salt, and an aqueous buffer solution.

7. The method for extracting one or more substances according to any one of claims 1 to 6, wherein the hydrophilic organic solvent is one or more solvents selected from the group consisting of methanol, ethanol, propanol, isopropanol, butanol, isobutanol, acetonitrile, dimethyl sulfoxide, dimethylformamide, dimethylacetamide, acetone, tetrahydrofuran and diethylene glycol.

8. The method for extracting one or more substances according to any one of claims 1 to 7, wherein the hydrophobic organic solvent is one or more solvents selected from the group consisting of chloroform, hexane, ether, diethyl ether, benzene, phenol, and isoamyl alcohol.

9. A method for identifying one or more substances in a fecal sample, and measuring a concentration, composition or proportion of the one or more substances after performing a method for extracting the one or more substances in the fecal sample, wherein the method for extracting the one or more substances in the fecal sample is the method according to any one of claims 1 to 8, and the method for identifying one or more substances in a fecal sample, and measuring a concentration, composition or proportion of the one or more substances, comprises:

step P of separating the one or more substances obtained in step C of the method for extracting by chromatography or capillary electrophoresis, and conducting mass spectrometry and/or nuclear magnetic resonance spectrometry to identify the one or more substances, and to measure the concentration, composition or proportion of the one or more substances.

10. A method for classifying a plurality of subjects into two or more clusters, comprising:

step Q of conducting the method according to claim 9 on each of fecal samples obtained from the plurality of subjects to identify two or more substances and to measure a concentration, composition or proportion of the two or more substances; and

step S of conducting cluster analysis on data of the concentration, composition or proportion of the two or more substances obtained in the step Q, and classifying the plurality of subjects into two or more clusters according to similarity of substance profiles.

11. The method according to claim 10, further comprising, between the step Q and the step S,

step R of standardizing the data of the concentration, composition or proportion of the two or more substances obtained in the step Q, wherein

the data standardized in the step R is used in the cluster analysis in the step S.