In one example, 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 example, 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 example, 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.

[0009d]

In one aspect, the present disclosure refers to a method for extracting one or more intestinal metabolites from a fecal sample derived from human, comprising:

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 following solvents (a) to (f) to obtain a suspension:

(a) a solvent comprising an aqueous solvent and a hydrophilic organic solvent, and not comprising a hydrophobic organic solvent, wherein a content of the aqueous solvent is in a range of 10 to 50% by weight, and a content of the hydrophilic organic solvent is in a range of 90 to 50% by weight;

(b) a solvent comprising an aqueous solvent, a hydrophilic organic solvent and a hydrophobic organic solvent, wherein a content of the aqueous solvent is in a range of 5 to 60% by weight, a content of the hydrophilic organic solvent is in a range of 10 to 70% by weight, and a content of the hydrophobic organic solvent is in a range of 10 to 70% by weight;

(c) a solvent comprising a hydrophilic organic solvent, and not comprising an aqueous solvent or a hydrophobic organic solvent;

(d) a solvent comprising a hydrophobic organic solvent, and not comprising an aqueous solvent or a hydrophilic organic solvent;

(e) a solvent comprising a hydrophilic organic solvent and a hydrophobic organic solvent, and not comprising an aqueous solvent; and

(f) 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 intestinal metabolites,

wherein the method further comprises before the step A-1, step X of drying the fecal sample derived from human, subjecting the 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-1.

[0009e]

In another aspect, the present disclosure refers to a method for identifying one or more intestinal metabolites in a fecal sample, and measuring a concentration, composition or proportion of the one or more intestinal metabolites after performing a method for extracting the one or more intestinal metabolites in the fecal sample, wherein the method for extracting the one or more intestinal metabolites in the fecal sample is the method according to any one of claims 1 to 6, and the method for identifying one or more intestinal metabolites in a fecal sample, and measuring a concentration,

composition or proportion of the one or more intestinal metabolites, comprises:

step P of separating the one or more intestinal metabolites 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 intestinal metabolites, and to measure the concentration, composition or proportion of the one or more intestinal metabolites.

[0009f]

In yet another aspect, the present 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 claim 7 on each of fecal samples obtained from the plurality of subjects to identify two or more intestinal metabolites and to measure a concentration, composition or proportion of the two or more intestinal metabolites; and

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

CLAIMS

1. A method for extracting one or more intestinal metabolites from a fecal sample derived from human, comprising:

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 following solvents (a) to (f) to obtain a suspension:

(a) a solvent comprising an aqueous solvent and a hydrophilic organic solvent, and not comprising a hydrophobic organic solvent, wherein a content of the aqueous solvent is in a range of 10 to 50% by weight, and a content of the hydrophilic organic solvent is in a range of 90 to 50% by weight;

(b) a solvent comprising an aqueous solvent, a hydrophilic organic solvent and a hydrophobic organic solvent, wherein a content of the aqueous solvent is in a range of 5 to 60% by weight, a content of the hydrophilic organic solvent is in a range of 10 to 70% by weight, and a content of the hydrophobic organic solvent is in a range of 10 to 70% by weight;

(c) a solvent comprising a hydrophilic organic solvent, and not comprising an aqueous solvent or a hydrophobic organic solvent;

(d) a solvent comprising a hydrophobic organic solvent, and not comprising an aqueous solvent or a hydrophilic organic solvent;

(e) a solvent comprising a hydrophilic organic solvent and a hydrophobic organic solvent, and not comprising an aqueous solvent; and

(f) 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 intestinal metabolites,

wherein the method further comprises before the step A-1, step X of drying the fecal sample derived from human, subjecting the 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-1.

2. The method for extracting one or more intestinal metabolites according to claim 1, wherein the solvent used in the step A-1 is the solvent (a), and the solvent (a) comprises water and methanol, and wherein a content of water in the solvent is in a range of 10 to 50% by weight, and a content of methanol is in a range of 90 to 50% by weight.

3. The method for extracting one or more intestinal metabolites according to claim 1, wherein the solvent used in the step A-1 is the solvent (c), and the solvent (c) comprises water, methanol, and chloroform, and wherein 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 intestinal metabolites according to claim 1, wherein the aqueous solvent is selected from the group consisting of water, an aqueous solution of salt, and an aqueous buffer solution.

5. The method for extracting one or more intestinal metabolites according to claim 1 or 4, 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.

6. The method for extracting one or more intestinal metabolites according to any one of claims 1, 4 and 5, 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.

7. A method for identifying one or more intestinal metabolites in a fecal sample, and measuring a concentration, composition or proportion of the one or more intestinal metabolites after performing a method for extracting the one or more intestinal metabolites in the fecal sample, wherein the method for extracting the one or more intestinal metabolites in the fecal sample is the method according to any one of claims 1 to 6, and the method for identifying one or more intestinal metabolites

in a fecal sample, and measuring a concentration, composition or proportion of the one or more intestinal metabolites, comprises:

step P of separating the one or more intestinal metabolites 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 intestinal metabolites, and to measure the concentration, composition or proportion of the one or more intestinal metabolites.

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

step Q of conducting the method according to claim 7 on each of fecal samples obtained from the plurality of subjects to identify two or more intestinal metabolites and to measure a concentration, composition or proportion of the two or more intestinal metabolites; and

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

9. The method according to claim 8, 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 intestinal metabolites obtained in the step Q, wherein

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