DESCRIPTION

Title of Invention: IMMUNOSTIMULATORY COMPOSITION

Technical Field

[0001] The present disclosure relates to an immunostimulatory composition.

Background Art

[0002] Immunostimulatory compositions comprising bacteria are known. For example, Patent Document 1 discloses an immunostimulatory food composition comprising a lactic acid bacterium that activates a pDC (plasmacytoid dendritic cell) and induces IFN-α (interferon α) production. Further, Patent Document 2 discloses a method for screening a lactic acid bacterium having an immunomodulatory activity, which comprises measuring the number of test lactic acid bacteria bound to uromodulin (Umod) protein, a lactic acid bacterium having an immunomodulatory activity obtained by the method, and an immunomodulatory composition comprising the lactic acid bacterium.

Citation List

Patent Document

[0003] [Patent Document 1] Japanese Unexamined Patent Application Publication No. 2017-201984

[Patent Document 2] Japanese Unexamined Patent Application Publication No. 2014-217372

Summary of Invention

Technical Problem

[0004] While multiple species of bacteria having immunostimulatory ability have been reported, the mechanisms by which these bacteria stimulate immunity and the properties they commonly possess remain largely unclear.

[0005] The present disclosure aims to provide an immunostimulatory composition.

Solution to Problem

[0006] The present inventors have found that a bacterium that is phagocytosed by a pDC has immunostimulatory ability. The present inventors have also found that a bacterium that binds to blood dendritic cell antigen 2 (BDCA2) has immunostimulatory ability.

[0007] The present disclosure relates to, for example, the following items.

[A0] A composition satisfying at least one selected from the group consisting of the following (1) to (4):

(1) that the composition comprises, as an active ingredient, a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC, and the composition is an immunostimulatory composition;

(2) that the composition comprises, as an active ingredient, a bacterium that binds to blood dendritic cell antigen 2 (BDCA2), and the composition is an immunostimulatory composition;

(3) that the composition comprises, as an active ingredient, a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the

bacterium satisfies the following Requirement X, and the composition is an immunostimulatory composition;

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium;

(4) that the composition comprises, as a bacterium, *Lactococcus lactis* subsp. *lactis* JCM 5805, wherein the *Lactococcus lactis* subsp. *lactis* JCM 5805 satisfies the following Requirement Y;

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[A1] An immunostimulatory composition comprising, as an active ingredient, a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC),

wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC.

[A2] An immunostimulatory composition comprising, as an active ingredient, a bacterium that binds to blood dendritic cell antigen 2

(BDCA2).

[A3] The composition according to [A0] or [A2], wherein the binding ability of the bacterium to BDCA2 is greater than the binding ability of *Lactococcus lactis* subsp. *lactis* ATCC 15577 to BDCA2.

[A4] The composition according to [A3], wherein the binding ability to BDCA2 is evaluated based on the proportion of bacteria bound to BDCA2 as evaluated in a binding assay.

[A5] An immunostimulatory composition comprising, as an active ingredient, a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the bacterium satisfies the following Requirement X:

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[A6] A composition comprising, as a bacterium, *Lactococcus lactis* subsp. *lactis* JCM 5805, wherein the *Lactococcus lactis* subsp. *lactis* JCM 5805 satisfies the following Requirement Y:

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are

pDCs pre-cultured in a serum-free medium.

[A7] The composition according to [A0] or [A6], wherein the composition is an immunostimulatory composition.

[A8] The composition according to any one of [A0] to [A5], wherein the bacterium is a lactic acid bacterium and/or an acetic acid bacterium.

[A9] The composition according to any one of [A0] to [A5], wherein the bacterium is one or two or more bacteria selected from the group consisting of bacteria of the genus *Lactococcus*, bacteria of the genus *Leuconostoc*, bacteria of the genus *Streptococcus*, bacteria of the genus *Enterococcus*, bacteria of the genus *Tetragenococcus*, bacteria of the genus *Oenococcus*, bacteria of the genus *Weissella*, bacteria of the genus *Bifidobacterium*, and bacteria of the genus *Lactobacillus*.

[A10] The composition according to any one of [A0] to [A5], wherein the bacterium is *Lactococcus lactis* subsp. *lactis*.

[A11] The composition according to any one of [A0] to [A5], wherein the bacterium is one or two or more bacteria selected from the group consisting of *Lactococcus plantarum* JCM 11056, *Lactococcus curvatus* JCM 1096, *Lactococcus murinus* JCM 1717, *Lactococcus parakefiri* JCM 8573, *Lactococcus paraplantarum* JCM 12533, *Leuconostoc carnosum* JCM 9695, *Streptococcus salivarius* JCM 5707, *Enterococcus lactis* JCM 30200, *Oenococcus oeni* JCM 6125, *Weissella paramesenteroides* JCM 9890, *Weissella viridescens* JCM 1174, *Bifidobacterium animalis* subsp. *animalis* JCM 1190, *Bifidobacterium animalis* subsp. *lactis* JCM 10602, *Bifidobacterium bifidum* JCM 1255, *Bifidobacterium longum* subsp. *infantis* JCM 1222, *Bifidobacterium pseudolongum* JCM 1205, *Lactococcus lactis* subsp. *lactis* ATCC 7963,

Lactococcus lactis subsp. *lactis* ATCC 7962, *Lactococcus lactis* subsp. *lactis* ATCC 15346, *Lactococcus lactis* subsp. *lactis* ATCC 13675, *Lactococcus lactis* subsp. *lactis* ATCC 12929, *Lactococcus lactis* subsp. *lactis* ATCC 11955, *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactococcus lactis* subsp. *lactis* ATCC 11007, and *Lactobacillus rhamnosus* CRL1505.

[A12] The composition according to any one of [A0] to [A11], wherein the bacterium comprises a dead bacterium.

[A13] The composition according to any one of [A0] to [A12], wherein the bacterium is a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) via binding to blood dendritic cell antigen 2 (BDCA2).

[A14] The composition according to [A13], wherein the phagocytosis of the bacterium by the pDC includes phagocytosis via binding to BDCA2 and phagocytosis not via binding to BDCA2, and the amount of phagocytosis of the bacterium via binding to BDCA2 is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 via binding to BDCA2.

[A15] The composition according to [A14], wherein the amount of phagocytosis not via binding to BDCA2 is the amount of phagocytosis of the bacterium by the pDC in a state where BDCA2 on the pDC cannot bind to a ligand.

[A16] The composition according to any one of [A13] to [A15], wherein the bacterium promotes production of IFN-α by the pDC via phagocytosis by the pDC.

[A17] The composition according to any one of [A0] to [A16], which is

a food composition, a pharmaceutical composition, or a feed.

[A18] The composition according to [A17], wherein the number of bacteria per unit package in the food composition, pharmaceutical composition, or feed is 1×10^8 or more.

[B0] A method for stimulating immunity of a subject, comprising feeding to the subject or administering to the subject in need thereof a bacterium satisfying at least one selected from the group consisting of the following (1) to (4):

(1) a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC;

(2) a bacterium that binds to blood dendritic cell antigen 2 (BDCA2);

(3) a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) and satisfies the following Requirement X;

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium;

(4) a bacterium that is *Lactococcus lactis* subsp. *lactis* JCM 5805 and satisfies the following Requirement Y;

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24

hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[B1] A method for stimulating immunity of a subject, comprising feeding to the subject or administering to the subject in need thereof a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC),

wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC.

[B2] A method for stimulating immunity of a subject, comprising feeding to the subject or administering to the subject in need thereof a bacterium that binds to blood dendritic cell antigen 2 (BDCA2).

[B3] The method according to [B0] or [B2], wherein the binding ability of the bacterium to BDCA2 is greater than the binding ability of *Lactococcus lactis* subsp. *lactis* ATCC 15577 to BDCA2.

[B4] The method according to [B3], wherein the binding ability to BDCA2 is evaluated based on the proportion of bacteria bound to BDCA2 as evaluated in a binding assay.

[B5] A method for stimulating immunity of a subject, comprising feeding to the subject or administering to the subject in need thereof a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the bacterium satisfies the following Requirement X:

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of

the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[B6] A method for stimulating immunity of a subject, comprising feeding to the subject or administering to the subject in need thereof *Lactococcus lactis* subsp. *lactis* JCM 5805 as a bacterium, wherein the *Lactococcus lactis* subsp. *lactis* JCM 5805 satisfies the following Requirement Y:

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[B7] The method according to any one of [B0] to [B5], wherein the bacterium is a lactic acid bacterium and/or an acetic acid bacterium.

[B8] The method according to any one of [B0] to [B5], wherein the bacterium is one or two or more bacteria selected from the group consisting of bacteria of the genus *Lactococcus*, bacteria of the genus *Leuconostoc*, bacteria of the genus *Streptococcus*, bacteria of the genus *Enterococcus*, bacteria of the genus *Tetragenococcus*, bacteria of the genus *Oenococcus*, bacteria of the genus *Weissella*, bacteria of the genus *Bifidobacterium*, and bacteria of the genus *Lactobacillus*.

[B9] The method according to any one of [B0] to [B5], wherein the bacterium is *Lactococcus lactis* subsp. *lactis*.

[B10] The method according to any one of [B0] to [B5], wherein the

bacterium is one or two or more bacteria selected from the group consisting of *Lactococcus plantarum* JCM 11056, *Lactococcus curvatus* JCM 1096, *Lactococcus murinus* JCM 1717, *Lactococcus parakefiri* JCM 8573, *Lactococcus paraplantarum* JCM 12533, *Leuconostoc carnosum* JCM 9695, *Streptococcus salivarius* JCM 5707, *Enterococcus lactis* JCM 30200, *Oenococcus oeni* JCM 6125, *Weissella paramesenteroides* JCM 9890, *Weissella viridescens* JCM 1174, *Bifidobacterium animalis* subsp. *animalis* JCM 1190, *Bifidobacterium animalis* subsp. *lactis* JCM 10602, *Bifidobacterium bifidum* JCM 1255, *Bifidobacterium longum* subsp. *infantis* JCM 1222, *Bifidobacterium pseudolongum* JCM 1205, *Lactococcus lactis* subsp. *lactis* ATCC 7963, *Lactococcus lactis* subsp. *lactis* ATCC 7962, *Lactococcus lactis* subsp. *lactis* ATCC 15346, *Lactococcus lactis* subsp. *lactis* ATCC 13675, *Lactococcus lactis* subsp. *lactis* ATCC 12929, *Lactococcus lactis* subsp. *lactis* ATCC 11955, *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactococcus lactis* subsp. *lactis* ATCC 11007, and *Lactobacillus rhamnosus* CRL1505.

[B11] The method according to any one of [B0] to [B10], wherein the bacterium comprises a dead bacterium.

[B12] The method according to any one of [B0] to [B11], wherein the bacterium is a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) via binding to blood dendritic cell antigen 2 (BDCA2).

[B13] The method according to [B12], wherein the phagocytosis of the bacterium by the pDC includes phagocytosis via binding to BDCA2 and phagocytosis not via binding to BDCA2, and the amount of phagocytosis

of the bacterium via binding to BDCA2 is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577via binding to BDCA2.

[B14] The method according to [B13], wherein the amount of phagocytosis not via binding to BDCA2 is the amount of phagocytosis of the bacterium by the pDC in a state where BDCA2 on the pDC cannot bind to a ligand.

[B15] The method according to any one of [B12] to [B14], wherein the bacterium promotes production of IFN-α by the pDC via phagocytosis by the pDC.

[B16] The method according to any one of [B0] to [B15], wherein the bacterium is comprised in a food composition, a pharmaceutical composition, or a feed.

[B17] The method according to [B16], wherein the number of the bacteria per unit package in the food composition, pharmaceutical composition, or feed is 1×10^8 or more.

[C0] A method for stimulating immunity of a subject, comprising feeding to the subject or administering to the subject in need thereof a composition comprising a bacterium satisfying at least one selected from the group consisting of the following (1) to (4):

(1) a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC;

(2) a bacterium that binds to blood dendritic cell antigen 2 (BDCA2);

(3) a bacterium that is phagocytosed by a plasmacytoid dendritic cell

(pDC) and satisfies the following Requirement X;

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium;

(4) a bacterium that is *Lactococcus lactis* subsp. *lactis* JCM 5805 and satisfies the following Requirement Y;

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[C1] A method for stimulating immunity of a subject, comprising feeding to the subject or administering to the subject in need thereof a composition comprising a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC),

wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC.

[C2] A method for stimulating immunity of a subject, comprising feeding to the subject or administering to the subject in need thereof a composition comprising a bacterium that binds to blood dendritic cell

antigen 2 (BDCA2).

[C3] The method according to [C0] or [C2], wherein the binding ability of the bacterium to BDCA2 is greater than the binding ability of *Lactococcus lactis* subsp. *lactis* ATCC 15577 to BDCA2.

[C4] The method according to [C3], wherein the binding ability to BDCA2 is evaluated based on the proportion of bacteria bound to BDCA2 as evaluated in a binding assay.

[C5] A method for stimulating immunity of a subject, comprising feeding to the subject or administering to the subject in need thereof a composition comprising a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the bacterium satisfies the following Requirement X:

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[C6] A method for stimulating immunity of a subject, comprising feeding to the subject or administering to the subject in need thereof a composition comprising *Lactococcus lactis* subsp. *lactis* JCM 5805 as a bacterium, wherein the *Lactococcus lactis* subsp. *lactis* JCM 5805 satisfies the following Requirement Y:

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24

hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[C7] The method according to any one of [C0] to [C5], wherein the bacterium is a lactic acid bacterium and/or an acetic acid bacterium.

[C8] The method according to any one of [C0] to [C5], wherein the bacterium is one or two or more bacteria selected from the group consisting of bacteria of the genus *Lactococcus*, bacteria of the genus *Leuconostoc*, bacteria of the genus *Streptococcus*, bacteria of the genus *Enterococcus*, bacteria of the genus *Tetragenococcus*, bacteria of the genus *Oenococcus*, bacteria of the genus *Weissella*, bacteria of the genus *Bifidobacterium*, and bacteria of the genus *Lactobacillus*.

[C9] The method according to any one of [C0] to [C5], wherein the bacterium is *Lactococcus lactis* subsp. *lactis*.

[C10] The method according to any one of [C0] to [C5], wherein the bacterium is one or two or more bacteria selected from the group consisting of *Lactococcus plantarum* JCM 11056, *Lactococcus curvatus* JCM 1096, *Lactococcus murinus* JCM 1717, *Lactococcus parakefiri* JCM 8573, *Lactococcus paraplantarum* JCM 12533, *Leuconostoc carnosum* JCM 9695, *Streptococcus salivarius* JCM 5707, *Enterococcus lactis* JCM 30200, *Oenococcus oeni* JCM 6125, *Weissella paramesenteroides* JCM 9890, *Weissella viridescens* JCM 1174, *Bifidobacterium animalis* subsp. *animalis* JCM 1190, *Bifidobacterium animalis* subsp. *lactis* JCM 10602, *Bifidobacterium bifidum* JCM 1255, *Bifidobacterium longum* subsp. *infantis* JCM 1222, *Bifidobacterium*

pseudolongum JCM 1205, *Lactococcus lactis* subsp. *lactis* ATCC 7963, *Lactococcus lactis* subsp. *lactis* ATCC 7962, *Lactococcus lactis* subsp. *lactis* ATCC 15346, *Lactococcus lactis* subsp. *lactis* ATCC 13675, *Lactococcus lactis* subsp. *lactis* ATCC 12929, *Lactococcus lactis* subsp. *lactis* ATCC 11955, *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactococcus lactis* subsp. *lactis* ATCC 11007, and *Lactobacillus rhamnosus* CRL1505.

[C11] The method according to any one of [C0] to [C10], wherein the bacterium comprises a dead bacterium.

[C12] The method according to any one of [C0] to [C11], wherein the bacterium is a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) via binding to blood dendritic cell antigen 2 (BDCA2).

[C13] The method according to [C12], wherein the phagocytosis of the bacterium by the pDC includes phagocytosis via binding to BDCA2 and phagocytosis not via binding to BDCA2, and the amount of phagocytosis of the bacterium via binding to BDCA2 is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 via binding to BDCA2.

[C14] The method according to [C13], wherein the amount of phagocytosis not via binding to BDCA2 is the amount of phagocytosis of the bacterium by the pDC in a state where BDCA2 on the pDC cannot bind to a ligand.

[C15] The method according to any one of [C12] to [C14], wherein the bacterium promotes production of IFN-α by the pDC via phagocytosis by the pDC.

[C16] The method according to any one of [C0] to [C15], wherein the composition is a food composition, a pharmaceutical composition, or a feed.

[C17] The method according to [C16], wherein the number of the bacteria per unit package in the food composition, pharmaceutical composition, or feed is 1×10^8 or more.

[D0] A bacterium for use in stimulation of immunity, satisfying at least one selected from the group consisting of the following (1) to (4):

(1) a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC;

(2) a bacterium that binds to blood dendritic cell antigen 2 (BDCA2);

(3) a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) and satisfies the following Requirement X;

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium;

(4) a bacterium that is *Lactococcus lactis* subsp. *lactis* JCM 5805 and satisfies the following Requirement Y;

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24

hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[D1] A bacterium for use in stimulation of immunity, which is a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC),

wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC.

[D2] A bacterium for use in stimulation of immunity, which is a bacterium that binds to blood dendritic cell antigen 2 (BDCA2).

[D3] The bacterium for use according to [D0] or [D2], wherein the binding ability of the bacterium to BDCA2 is greater than the binding ability of *Lactococcus lactis* subsp. *lactis* ATCC 15577 to BDCA2.

[D4] The bacterium for use according to [D3], wherein the binding ability to BDCA2 is evaluated based on the proportion of bacteria bound to BDCA2 as evaluated in a binding assay.

[D5] A bacterium for use in stimulation of immunity, which is a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the bacterium satisfies the following Requirement X:

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[D6] *Lactococcus lactis* subsp. *lactis* JCM 5805 for use in stimulation of immunity, which is a bacterium that is *Lactococcus lactis* subsp. *lactis* JCM 5805, wherein the *Lactococcus lactis* subsp. *lactis* JCM 5805 satisfies the following Requirement Y:

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[D7] The bacterium for use according to any one of [D0] to [D5], wherein the bacterium is a lactic acid bacterium and/or an acetic acid bacterium.

[D8] The bacterium for use according to any one of [D0] to [D5], wherein the bacterium is one or two or more bacteria selected from the group consisting of bacteria of the genus *Lactococcus*, bacteria of the genus *Leuconostoc*, bacteria of the genus *Streptococcus*, bacteria of the genus *Enterococcus*, bacteria of the genus *Tetragenococcus*, bacteria of the genus *Oenococcus*, bacteria of the genus *Weissella*, bacteria of the genus *Bifidobacterium*, and bacteria of the genus *Lactobacillus*.

[D9] The bacterium for use according to any one of [D0] to [D5], wherein the bacterium is *Lactococcus lactis* subsp. *lactis*.

[D10] The bacterium for use according to any one of [D0] to [D5], wherein the bacterium is one or two or more bacteria selected from the group consisting of *Lactococcus plantarum* JCM 11056, *Lactococcus*

curvatus JCM 1096, *Lactococcus murinus* JCM 1717, *Lactococcus parakefiri* JCM 8573, *Lactococcus paraplantarum* JCM 12533, *Leuconostoc carnosum* JCM 9695, *Streptococcus salivarius* JCM 5707, *Enterococcus lactis* JCM 30200, *Oenococcus oeni* JCM 6125, *Weissella parameseenteroides* JCM 9890, *Weissella viridescens* JCM 1174, *Bifidobacterium animalis* subsp. *animalis* JCM 1190, *Bifidobacterium animalis* subsp. *lactis* JCM 10602, *Bifidobacterium bifidum* JCM 1255, *Bifidobacterium longum* subsp. *infantis* JCM 1222, *Bifidobacterium pseudolongum* JCM 1205, *Lactococcus lactis* subsp. *lactis* ATCC 7963, *Lactococcus lactis* subsp. *lactis* ATCC 7962, *Lactococcus lactis* subsp. *lactis* ATCC 15346, *Lactococcus lactis* subsp. *lactis* ATCC 13675, *Lactococcus lactis* subsp. *lactis* ATCC 12929, *Lactococcus lactis* subsp. *lactis* ATCC 11955, *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactococcus lactis* subsp. *lactis* ATCC 11007, and *Lactobacillus rhamnosus* CRL1505.

[D11] The bacterium for use according to any one of [D0] to [D10], wherein the bacterium comprises a dead bacterium.

[D12] The bacterium for use according to any one of [D0] to [D11], wherein the bacterium is a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) via binding to blood dendritic cell antigen 2 (BDCA2).

[D13] The bacterium for use according to [D12], wherein the phagocytosis of the bacterium by the pDC includes phagocytosis via binding to BDCA2 and phagocytosis not via binding to BDCA2, and the amount of phagocytosis of ithe bacterium via binding to BDCA2 is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp.

lactis ATCC 15577via binding to BDCA2.

[D14] The bacterium for use according to [D13], wherein the amount of phagocytosis not via binding to BDCA2 is the amount of phagocytosis of the bacterium by the pDC in a state where BDCA2 on the pDC cannot bind to a ligand.

[D15] The bacterium for use according to any one of [D12] to [D14], wherein the bacterium promotes production of IFN-α by the pDC via phagocytosis by the pDC.

[D16] The bacterium for use according to any one of [D0] to [D15], wherein the bacterium is comprised in a food composition, a pharmaceutical composition, or a feed.

[D17] The bacterium for use according to [D16], wherein the number of the bacteria per unit package in the food composition, pharmaceutical composition, or feed is 1×10^8 or more.

[E0] A composition for use in stimulation of immunity, comprising a bacterium satisfying at least one selected from the group consisting of the following (1) to (4):

(1) a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC;

(2) a bacterium that binds to blood dendritic cell antigen 2 (BDCA2);

(3) a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) and satisfies the following Requirement X;

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10

μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium;

(4) a bacterium that is *Lactococcus lactis* subsp. *lactis* JCM 5805 and satisfies the following Requirement Y;

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[E1] A composition for use in stimulation of immunity, comprising a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC),

wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC.

[E2] A composition for use in stimulation of immunity, comprising a bacterium that binds to blood dendritic cell antigen 2 (BDCA2).

[E3] The composition for use according to [E0] or [E2], wherein the binding ability of the bacterium to BDCA2 is greater than the binding ability of *Lactococcus lactis* subsp. *lactis* ATCC 15577 to BDCA2.

[E4] The composition for use according to [E3], wherein the binding ability to BDCA2 is evaluated based on the proportion of bacteria bound to BDCA2 as evaluated in a binding assay.

[E5] A composition for use in stimulation of immunity, comprising a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the bacterium satisfies the following Requirement X:

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[E6] A composition for use in stimulation of immunity, comprising *Lactococcus lactis* subsp. *lactis* JCM 5805 as a bacterium, wherein the *Lactococcus lactis* subsp. *lactis* JCM 5805 satisfies the following Requirement Y:

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[E7] The composition for use according to any one of [E0] to [E5], wherein the bacterium is a lactic acid bacterium and/or an acetic acid bacterium.

[E8] The composition for use according to any one of [E0] to [E5], wherein the bacterium is one or two or more bacteria selected from the group consisting of bacteria of the genus *Lactococcus*, bacteria of the

genus *Leuconostoc*, bacteria of the genus *Streptococcus*, bacteria of the genus *Enterococcus*, bacteria of the genus *Tetragenococcus*, bacteria of the genus *Oenococcus*, bacteria of the genus *Weissella*, bacteria of the genus *Bifidobacterium*, and bacteria of the genus *Lactobacillus*.

[E9] The composition for use according to any one of [E0] to [E5], wherein the bacterium is *Lactococcus lactis* subsp. *lactis*.

[E10] The composition for use according to any one of [E0] to [E5], wherein the bacterium is one or two or more bacteria selected from the group consisting of *Lactococcus plantarum* JCM 11056, *Lactococcus curvatus* JCM 1096, *Lactococcus murinus* JCM 1717, *Lactococcus parakefiri* JCM 8573, *Lactococcus paraplantarum* JCM 12533, *Leuconostoc carnosum* JCM 9695, *Streptococcus salivarius* JCM 5707, *Enterococcus lactis* JCM 30200, *Oenococcus oeni* JCM 6125, *Weissella paramesenteroides* JCM 9890, *Weissella viridescens* JCM 1174, *Bifidobacterium animalis* subsp. *animalis* JCM 1190, *Bifidobacterium animalis* subsp. *lactis* JCM 10602, *Bifidobacterium bifidum* JCM 1255, *Bifidobacterium longum* subsp. *infantis* JCM 1222, *Bifidobacterium pseudolongum* JCM 1205, *Lactococcus lactis* subsp. *lactis* ATCC 7963, *Lactococcus lactis* subsp. *lactis* ATCC 7962, *Lactococcus lactis* subsp. *lactis* ATCC 15346, *Lactococcus lactis* subsp. *lactis* ATCC 13675, *Lactococcus lactis* subsp. *lactis* ATCC 12929, *Lactococcus lactis* subsp. *lactis* ATCC 11955, *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactococcus lactis* subsp. *lactis* ATCC 11007, and *Lactobacillus rhamnosus* CRL1505.

[E11] The composition for use according to any one of [E0] to [E10], wherein the bacterium comprises a dead bacterium.

[E12] The composition for use according to any one of [E0] to [E11], wherein the bacterium is a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) via binding to blood dendritic cell antigen 2 (BDCA2).

[E13] The composition for use according to [E12], wherein the phagocytosis of the bacterium by the pDC includes phagocytosis via binding to BDCA2 and phagocytosis not via binding to BDCA2, and the amount of phagocytosis of the bacterium via binding to BDCA2 is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577via binding to BDCA2.

[E14] The composition for use according to [E13], wherein the amount of phagocytosis not via binding to BDCA2 is the amount of phagocytosis of the bacterium by the pDC in a state where BDCA2 on the pDC cannot bind to a ligand.

[E15] The composition for use according to any one of [E12] to [E14], wherein the bacterium promotes production of IFN-α by the pDC via phagocytosis by the pDC.

[E16] The composition for use according to any one of [E0] to [E15], which is a food composition, a pharmaceutical composition, or a feed.

[E17] The composition for use according to [E16], wherein the number of the bacteria per unit package in the food composition, pharmaceutical composition, or feed is 1×10^8 or more.

[F0] Use of a bacterium satisfying at least one selected from the group consisting of the following (1) to (4) in stimulating immunity:

(1) a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the amount of phagocytosis of the bacterium by the pDC

is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC;

(2) a bacterium that binds to blood dendritic cell antigen 2 (BDCA2);

(3) a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) and satisfies the following Requirement X;

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium;

(4) a bacterium that is *Lactococcus lactis* subsp. *lactis* JCM 5805 and satisfies the following Requirement Y;

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[F1] Use of a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) in stimulating immunity,

wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC.

[F2] Use of a bacterium that binds to blood dendritic cell antigen 2

(BDCA2) in stimulating immunity.

[F3] The use according to [F0] or [F2], wherein the binding ability of the bacterium to BDCA2 is greater than the binding ability of *Lactococcus lactis* subsp. *lactis* ATCC 15577 to BDCA2.

[F4] The use according to [F3], wherein the binding ability to BDCA2 is evaluated based on the proportion of bacteria bound to BDCA2 as evaluated in a binding assay.

[F5] Use of a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) in stimulating immunity, wherein the bacterium satisfies the following Requirement X:

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[F6] Use of *Lactococcus lactis* subsp. *lactis* JCM 5805, which is a bacterium, in stimulating immunity, wherein the *Lactococcus lactis* subsp. *lactis* JCM 5805 satisfies the following Requirement Y:

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[F7] The use according to any one of [F0] to [F5], wherein the bacterium is a lactic acid bacterium and/or an acetic acid bacterium.

[F8] The use according to any one of [F0] to [F5], wherein the bacterium is one or two or more bacteria selected from the group consisting of bacteria of the genus *Lactococcus*, bacteria of the genus *Leuconostoc*, bacteria of the genus *Streptococcus*, bacteria of the genus *Enterococcus*, bacteria of the genus *Tetragenococcus*, bacteria of the genus *Oenococcus*, bacteria of the genus *Weissella*, bacteria of the genus *Bifidobacterium*, and bacteria of the genus *Lactobacillus*.

[F9] The use according to any one of [F0] to [F5], wherein the bacterium is *Lactococcus lactis* subsp. *lactis*.

[F10] The use according to any one of [F0] to [F5], wherein the bacterium is one or two or more bacteria selected from the group consisting of *Lactococcus plantarum* JCM 11056, *Lactococcus curvatus* JCM 1096, *Lactococcus murinus* JCM 1717, *Lactococcus parakefiri* JCM 8573, *Lactococcus paraplantarum* JCM 12533, *Leuconostoc carnosum* JCM 9695, *Streptococcus salivarius* JCM 5707, *Enterococcus lactis* JCM 30200, *Oenococcus oeni* JCM 6125, *Weissella paramesenteroides* JCM 9890, *Weissella viridescens* JCM 1174, *Bifidobacterium animalis* subsp. *animalis* JCM 1190, *Bifidobacterium animalis* subsp. *lactis* JCM 10602, *Bifidobacterium bifidum* JCM 1255, *Bifidobacterium longum* subsp. *infantis* JCM 1222, *Bifidobacterium pseudolongum* JCM 1205, *Lactococcus lactis* subsp. *lactis* ATCC 7963, *Lactococcus lactis* subsp. *lactis* ATCC 7962, *Lactococcus lactis* subsp. *lactis* ATCC 15346, *Lactococcus lactis* subsp. *lactis* ATCC 13675, *Lactococcus lactis* subsp. *lactis* ATCC 12929, *Lactococcus lactis* subsp.

lactis ATCC 11955, *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactococcus lactis* subsp. *lactis* ATCC 11007, and *Lactobacillus rhamnosus* CRL1505.

[F11] The use according to any one of [F0] to [F10], wherein the bacterium comprises a dead bacterium.

[F12] The use according to any one of [F0] to [F11], wherein the bacterium is a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) via binding to blood dendritic cell antigen 2 (BDCA2).

[F13] The use according to [F12], wherein the phagocytosis of the bacterium by the pDC includes phagocytosis via binding to BDCA2 and phagocytosis not via binding to BDCA2, and the amount of phagocytosis of the bacterium via binding to BDCA2 is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 via binding to BDCA2.

[F14] The use according to [F13], wherein the amount of phagocytosis not via binding to BDCA2 is the amount of phagocytosis of the bacterium by the pDC in a state where BDCA2 on the pDC cannot bind to a ligand.

[F15] The use according to any one of [F12] to [F14], wherein the bacterium promotes production of IFN-α by the pDC via phagocytosis by the pDC.

[F16] The use according to any one of [F0] to [F15], wherein the bacterium is comprised in a food composition, a pharmaceutical composition, or a feed.

[F17] The use according to [F16], wherein the number of the bacteria per

unit package in the food composition, pharmaceutical composition, or feed is 1×10^8 or more.

[F18] The use according to any one of [F0] to [F17], which is a non-therapeutic use.

[G0] Use of a composition comprising a bacterium satisfying at least one selected from the group consisting of the following (1) to (4) in stimulating immunity:

(1) a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC;

(2) a bacterium that binds to blood dendritic cell antigen 2 (BDCA2);

(3) a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) and satisfies the following Requirement X;

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium;

(4) a bacterium that is *Lactococcus lactis* subsp. *lactis* JCM 5805 and satisfies the following Requirement Y;

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the

Lactococcus lactis subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[G1] Use of a composition comprising a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) in stimulating immunity,

wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC.

[G2] Use of a composition comprising a bacterium that binds to blood dendritic cell antigen 2 (BDCA2) in stimulating immunity.

[G3] The use according to [G0] or [G2], wherein the binding ability of the bacterium to BDCA2 is greater than the binding ability of *Lactococcus lactis* subsp. *lactis* ATCC 15577 to BDCA2.

[G4] The use according to [G3], wherein the binding ability to BDCA2 is evaluated based on the proportion of bacteria bound to BDCA2 as evaluated in a binding assay.

[G5] Use of a composition comprising a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) in stimulating immunity, wherein the bacterium satisfies the following Requirement X:

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[G6] Use of a composition comprising *Lactococcus lactis* subsp. *lactis*

JCM 5805, which is a bacterium, in stimulating immunity, wherein the *Lactococcus lactis* subsp. *lactis* JCM 5805 satisfies the following Requirement Y:

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[G7] The use according to any one of [G0] to [G5], wherein the bacterium is a lactic acid bacterium and/or an acetic acid bacterium.

[G8] The use according to any one of [G0] to [G5], wherein the bacterium is one or two or more bacteria selected from the group consisting of bacteria of the genus *Lactococcus*, bacteria of the genus *Leuconostoc*, bacteria of the genus *Streptococcus*, bacteria of the genus *Enterococcus*, bacteria of the genus *Tetragenococcus*, bacteria of the genus *Oenococcus*, bacteria of the genus *Weissella*, bacteria of the genus *Bifidobacterium*, and bacteria of the genus *Lactobacillus*.

[G9] The use according to any one of [G0] to [G5], wherein the bacterium is *Lactococcus lactis* subsp. *lactis*.

[G10] The use according to any one of [G0] to [G5], wherein the bacterium is one or two or more bacteria selected from the group consisting of *Lactococcus plantarum* JCM 11056, *Lactococcus curvatus* JCM 1096, *Lactococcus murinus* JCM 1717, *Lactococcus parakefiri* JCM 8573, *Lactococcus paraplantarum* JCM 12533, *Leuconostoc*

carnosum JCM 9695, *Streptococcus salivarius* JCM 5707, *Enterococcus lactis* JCM 30200, *Oenococcus oeni* JCM 6125, *Weissella paramesenteroides* JCM 9890, *Weissella viridescens* JCM 1174, *Bifidobacterium animalis* subsp. *animalis* JCM 1190, *Bifidobacterium animalis* subsp. *lactis* JCM 10602, *Bifidobacterium bifidum* JCM 1255, *Bifidobacterium longum* subsp. *infantis* JCM 1222, *Bifidobacterium pseudolongum* JCM 1205, *Lactococcus lactis* subsp. *lactis* ATCC 7963, *Lactococcus lactis* subsp. *lactis* ATCC 7962, *Lactococcus lactis* subsp. *lactis* ATCC 15346, *Lactococcus lactis* subsp. *lactis* ATCC 13675, *Lactococcus lactis* subsp. *lactis* ATCC 12929, *Lactococcus lactis* subsp. *lactis* ATCC 11955, *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactococcus lactis* subsp. *lactis* ATCC 11007, and *Lactobacillus rhamnosus* CRL1505.

[G11] The use according to any one of [G0] to [G10], wherein the bacterium comprises a dead bacterium.

[G12] The use according to any one of [G0] to [G11], wherein the bacterium is a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) via binding to blood dendritic cell antigen 2 (BDCA2).

[G13] The use according to [G12], wherein the phagocytosis of the bacterium by the pDC includes phagocytosis via binding to BDCA2 and phagocytosis not via binding to BDCA2, and the amount of phagocytosis of the bacterium via binding to BDCA2 is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 via binding to BDCA2.

[G14] The use according to [G13], wherein the amount of phagocytosis

not via binding to BDCA2 is the amount of phagocytosis of the bacterium by the pDC in a state where BDCA2 on the pDC cannot bind to a ligand.

[G15] The use according to any one of [G12] to [G14], wherein the bacterium promotes production of IFN-α by the pDC via phagocytosis by the pDC.

[G16] The use according to any one of [G0] to [G15], wherein the composition is a food composition, a pharmaceutical composition, or a feed.

[G17] The use according to [G16], wherein the number of the bacteria per unit package in the food composition, pharmaceutical composition, or feed is 1×10^8 or more.

[G18] The use according to any one of [G0] to [G17], which is a non-therapeutic use.

[H0] Use of a bacterium satisfying at least one selected from the group consisting of the following (1) to (4) in the manufacture of an immunostimulatory composition:

(1) a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC;

(2) a bacterium that binds to blood dendritic cell antigen 2 (BDCA2);

(3) a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) and satisfies the following Requirement X;

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10

μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium;

(4) a bacterium that is *Lactococcus lactis* subsp. *lactis* JCM 5805 and satisfies the following Requirement Y;

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[H1] Use of a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) in the manufacture of an immunostimulatory composition,

wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC.

[H2] Use of a bacterium that binds to blood dendritic cell antigen 2 (BDCA2) in the manufacture of an immunostimulatory composition.

[H3] The use according to [H0] or [H2], wherein the binding ability of the bacterium to BDCA2 is greater than the binding ability of *Lactococcus lactis* subsp. *lactis* ATCC 15577 to BDCA2.

[H4] The use according to [H3], wherein the binding ability to BDCA2 is evaluated based on the proportion of bacteria bound to BDCA2 as

evaluated in a binding assay.

[H5] Use of a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) in the manufacture of an immunostimulatory composition, wherein the bacterium satisfies the following Requirement X:

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[H6] Use of *Lactococcus lactis* subsp. *lactis* JCM 5805, which is a bacterium, in the manufacture of an immunostimulatory composition, wherein the *Lactococcus lactis* subsp. *lactis* JCM 5805 satisfies the following Requirement Y:

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[H7] The use according to any one of [H0] to [H5], wherein the bacterium is a lactic acid bacterium and/or an acetic acid bacterium.

[H8] The use according to any one of [H0] to [H5], wherein the bacterium is one or two or more bacteria selected from the group

consisting of bacteria of the genus *Lactococcus*, bacteria of the genus *Leuconostoc*, bacteria of the genus *Streptococcus*, bacteria of the genus *Enterococcus*, bacteria of the genus *Tetragenococcus*, bacteria of the genus *Oenococcus*, bacteria of the genus *Weissella*, bacteria of the genus *Bifidobacterium*, and bacteria of the genus *Lactobacillus*.

[H9] The use according to any one of [H0] to [H5], wherein the bacterium is *Lactococcus lactis* subsp. *lactis*.

[H10] The use according to any one of [H0] to [H5], wherein the bacterium is one or two or more bacteria selected from the group consisting of *Lactococcus plantarum* JCM 11056, *Lactococcus curvatus* JCM 1096, *Lactococcus murinus* JCM 1717, *Lactococcus parakefiri* JCM 8573, *Lactococcus paraplantarum* JCM 12533, *Leuconostoc carnosum* JCM 9695, *Streptococcus salivarius* JCM 5707, *Enterococcus lactis* JCM 30200, *Oenococcus oeni* JCM 6125, *Weissella paramesenteroides* JCM 9890, *Weissella viridescens* JCM 1174, *Bifidobacterium animalis* subsp. *animalis* JCM 1190, *Bifidobacterium animalis* subsp. *lactis* JCM 10602, *Bifidobacterium bifidum* JCM 1255, *Bifidobacterium longum* subsp. *infantis* JCM 1222, *Bifidobacterium pseudolongum* JCM 1205, *Lactococcus lactis* subsp. *lactis* ATCC 7963, *Lactococcus lactis* subsp. *lactis* ATCC 7962, *Lactococcus lactis* subsp. *lactis* ATCC 15346, *Lactococcus lactis* subsp. *lactis* ATCC 13675, *Lactococcus lactis* subsp. *lactis* ATCC 12929, *Lactococcus lactis* subsp. *lactis* ATCC 11955, *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactococcus lactis* subsp. *lactis* ATCC 11007, and *Lactobacillus rhamnosus* CRL1505.

[H11] The use according to any one of [H0] to [H10], wherein the

bacterium comprises a dead bacterium.

[H12] The use according to any one of [H0] to [H11], wherein the bacterium is a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) via binding to blood dendritic cell antigen 2 (BDCA2).

[H13] The use according to [H12], wherein the phagocytosis of the bacterium by the pDC includes phagocytosis via binding to BDCA2 and phagocytosis not via binding to BDCA2, and the amount of phagocytosis of the bacterium via binding to BDCA2 is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 via binding to BDCA2.

[H14] The use according to [H13], wherein the amount of phagocytosis not via binding to BDCA2 is the amount of phagocytosis of the bacterium by the pDC in a state where BDCA2 on the pDC cannot bind to a ligand.

[H15] The use according to any one of [H12] to [H14], wherein the bacterium promotes production of IFN-α by the pDC via phagocytosis by the pDC.

[H16] The use according to any one of [H0] to [H15], wherein the composition is a food composition, a pharmaceutical composition, or a feed.

[H17] The use according to [H16], wherein the number of the bacteria per unit package in the food composition, pharmaceutical composition, or feed is 1×10^8 or more.

[I1] A method for screening a bacterium having immunostimulatory ability, comprising a step of screening the bacterium having the

immunostimulatory ability using binding to blood dendritic cell antigen 2 (BDCA2) as an indicator.

[I2] The method according to [I1], wherein the screening step is performed by a binding assay between a candidate bacterium and BDCA2.

[I3] The method according to [I1] or [I2], wherein the bacterium according to any one of [A0] to [A18], [B0] to [B17], [C0] to [C17], [D0] to [D17], [E0] to [E17], [F0] to [F18], [G0] to [G18], and [H0] to [H17] is selected.

[I4] The composition, method, bacterium for use, composition for use, or use according to any one of [A0] to [A18], [B0] to [B17], [C0] to [C17], [D0] to [D17], [E0] to [E17], [F0] to [F18], [G0] to [G18], and [H0] to [H17], wherein the bacterium is one selected by the method according to [I1] or [I2].

[J1] A method for activating a pDC, characterized by binding a bacterium to BDCA2.

[J2] A method for activating a pDC, comprising feeding to a subject or administering to a subject in need thereof a bacterium that binds to BDCA2.

[J3] The method according to [J1] or [J2], comprising the bacterium being phagocytosed by the pDC via binding to BDCA2.

[J4] A composition comprising a bacterium, for use in the method according to any one of [J1] to [J3].

[J5] The method according to any one of [J1] to [J3] or the composition according to [J4], wherein the bacterium is a lactic acid bacterium and/or an acetic acid bacterium.

[J6] The method according to any one of [J1] to [J3] or the composition according to [J4] or [J5], wherein the bacterium is one or two or more bacteria selected from the group consisting of bacteria of the genus *Lactococcus*, bacteria of the genus *Leuconostoc*, bacteria of the genus *Streptococcus*, bacteria of the genus *Enterococcus*, bacteria of the genus *Tetragenococcus*, bacteria of the genus *Oenococcus*, bacteria of the genus *Weissella*, bacteria of the genus *Bifidobacterium*, and bacteria of the genus *Lactobacillus*.

[J7] The method according to any one of [J1] to [J3] or the composition according to any one of [J4] to [J6], wherein the bacterium is *Lactococcus lactis* subsp. *lactis*.

[J8] The method according to any one of [J1] to [J3] or the composition according to any one of [J4] to [J7], wherein the bacterium is one or two or more bacteria selected from the group consisting of *Lactococcus plantarum* JCM 11056, *Lactococcus curvatus* JCM 1096, *Lactococcus murinus* JCM 1717, *Lactococcus parakefiri* JCM 8573, *Lactococcus paraplantarum* JCM 12533, *Leuconostoc carnosum* JCM 9695, *Streptococcus salivarius* JCM 5707, *Enterococcus lactis* JCM 30200, *Oenococcus oeni* JCM 6125, *Weissella paramesenteroides* JCM 9890, *Weissella viridescens* JCM 1174, *Bifidobacterium animalis* subsp. *animalis* JCM 1190, *Bifidobacterium animalis* subsp. *lactis* JCM 10602, *Bifidobacterium bifidum* JCM 1255, *Bifidobacterium longum* subsp. *infantis* JCM 1222, *Bifidobacterium pseudolongum* JCM 1205, *Lactococcus lactis* subsp. *lactis* ATCC 7963, *Lactococcus lactis* subsp. *lactis* ATCC 7962, *Lactococcus lactis* subsp. *lactis* ATCC 15346, *Lactococcus lactis* subsp. *lactis* ATCC 13675, *Lactococcus lactis* subsp.

lactis ATCC 12929, *Lactococcus lactis* subsp. *lactis* ATCC 11955, *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactococcus lactis* subsp. *lactis* ATCC 11007, and *Lactobacillus rhamnosus* CRL1505.

[J9] The method according to any one of [J1] to [J3] or the composition according to any one of [J4] to [J8], wherein the bacterium comprises a dead bacterium.

[K1] A method for enhancing immunostimulatory ability and/or susceptibility to phagocytosis by a plasmacytoid dendritic cell (pDC) of a bacterium, comprising a step of culturing the bacterium in M17 medium supplemented with glucose at a final concentration of 0.1 to 10 mass%.

[K2] The method according to [K1], wherein the bacterium is a lactic acid bacterium and/or an acetic acid bacterium.

[K3] The method according to [K1] or [K2], wherein the bacterium is one or two or more bacteria selected from the group consisting of bacteria of the genus *Lactococcus*, bacteria of the genus *Leuconostoc*, bacteria of the genus *Streptococcus*, bacteria of the genus *Enterococcus*, bacteria of the genus *Tetragenococcus*, bacteria of the genus *Oenococcus*, bacteria of the genus *Weissella*, bacteria of the genus *Bifidobacterium*, and bacteria of the genus *Lactobacillus*.

[K4] The method according to any one of [K1] to [K3], wherein the bacterium is *Lactococcus lactis* subsp. *lactis*.

[K5] The method according to any one of [K1] to [K4], wherein the bacterium is one or two or more bacteria selected from the group consisting of *Lactococcus plantarum* JCM 11056, *Lactococcus curvatus* JCM 1096, *Lactococcus murinus* JCM 1717, *Lactococcus parakefiri*

JCM 8573, *Lactococcus paraplantarum* JCM 12533, *Leuconostoc carnosum* JCM 9695, *Streptococcus salivarius* JCM 5707, *Enterococcus lactis* JCM 30200, *Oenococcus oeni* JCM 6125, *Weissella paramesenteroides* JCM 9890, *Weissella viridescens* JCM 1174, *Bifidobacterium animalis* subsp. *animalis* JCM 1190, *Bifidobacterium animalis* subsp. *lactis* JCM 10602, *Bifidobacterium bifidum* JCM 1255, *Bifidobacterium longum* subsp. *infantis* JCM 1222, *Bifidobacterium pseudolongum* JCM 1205, *Lactococcus lactis* subsp. *lactis* ATCC 7963, *Lactococcus lactis* subsp. *lactis* ATCC 7962, *Lactococcus lactis* subsp. *lactis* ATCC 15346, *Lactococcus lactis* subsp. *lactis* ATCC 13675, *Lactococcus lactis* subsp. *lactis* ATCC 12929, *Lactococcus lactis* subsp. *lactis* ATCC 11955, *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactococcus lactis* subsp. *lactis* ATCC 11007, and *Lactobacillus rhamnosus* CRL1505.

[K6] The method according to any one of [K1] to [K5], wherein the bacterium is *Lactococcus lactis* subsp. *lactis* JCM 5805.

[K7] A bacterium having enhanced immunostimulatory ability and/or susceptibility to phagocytosis by a plasmacytoid dendritic cell (pDC) by the method according to any one of [K1] to [K6].

[L1] A method for producing an immunostimulatory composition comprising *Lactococcus lactis* subsp. *lactis* JCM 5805 as an active ingredient, comprising a step of culturing *Lactococcus lactis* subsp. *lactis* JCM 5805 in M17 medium supplemented with glucose at a final concentration of 0.1 to 10 mass%.

[L2] The production method according to [L1], wherein the composition according to any one of [A0] to [A17], [C0] to [C17], [E0] to [E17], [G0]

to [G18], and [H0] to [H17] is produced.

[L3] The composition, method, composition for use, or use according to any one of [A0] to [A17], [C0] to [C17], [E0] to [E17], [G0] to [G18], and [H0] to [H17], wherein the composition is a composition produced by the production method according to [L1].

Advantageous Effects of Invention

[0008] According to the present invention, an immunostimulatory composition can be provided. For example, in the immunostimulatory composition of the present invention, the bacterium as an active ingredient can stimulate immunity via being phagocytosed by a plasmacytoid dendritic cell (pDC). For example, in the immunostimulatory composition of the present invention, the bacterium as an active ingredient can stimulate immunity via binding to BDCA2 on the surface of a plasmacytoid dendritic cell (pDC). For example, in the immunostimulatory composition of the present invention, the bacterium as an active ingredient can stimulate immunity by being phagocytosed by dendritic cells via binding to BDCA2 on the surface of pDCs and thereby activating the pDCs.

[0009] According to the present invention, a method for screening a bacterium having immunostimulatory ability can be provided. For example, in the screening method of the present invention, using binding of a bacterium to BDCA2 as an indicator, a bacterium that binds to BDCA2 can be selected as a bacterium that may have immunostimulatory ability.

Brief Description of Drawings

[0010] [FIG. 1] A diagram showing the fluorescence detection area 22

hours after addition of LC-Plasma in Example 1.

[FIG. 2] A diagram showing the measurement results of IFN-α concentration in the culture supernatant collected 22 hours after addition of LC-Plasma in Example 1.

[FIG. 3] A diagram showing the fluorescence detection area 22 hours after addition of lactic acid bacteria for the eight species of lactic acid bacteria tested in Example 2.

[FIG. 4] A diagram showing the measurement results of IFN-α concentration in the culture supernatant collected 22 hours after addition of lactic acid bacteria for the eight species of lactic acid bacteria tested in Example 2.

[FIG. 5] A diagram showing the correlation between the fluorescence detection area on the horizontal axis and the IFN-α concentration in the culture supernatant on the vertical axis for the eight species of lactic acid bacteria tested in Example 2.

[FIG. 6] A diagram showing the fluorescence detection area 22 hours after addition of LC-Plasma for the PL group, PL+Isotype group, and PL+anti-BDCA2 group in Example 3.

[FIG. 7] A diagram showing the measurement results of IFN-α concentration in the culture supernatant collected 22 hours after addition of LC-Plasma for the PL group, PL+Isotype group, and PL+anti-BDCA2 group in Example 3.

[FIG. 8] A diagram showing the fluorescence detection area 22 hours after addition of various lactic acid bacteria for the Isotype group and anti-BDCA2 group in Example 4.

[FIG. 9] A diagram showing the measurement results of IFN-α

concentration in the culture supernatant collected 22 hours after addition of various lactic acid bacteria for the Isotype group and anti-BDCA2 group in Example 4.

[FIG. 10] A diagram showing the correlation between the amount of phagocytosis via binding to BDCA2 on the horizontal axis and the IFN-α concentration in the culture supernatant on the vertical axis for various lactic acid bacteria.

[FIG. 11] A diagram showing the proportion of LC-Plasma bound to BDCA2 under conditions where the BDCA2 concentration is 1, 5, 10, 20, or 50 μg/mL, as evaluated by BDCA2 binding assay in Example 5.

[FIG. 12] A diagram showing the results of measuring the number of viable cells by Cell Counting Kit-8 after addition of DMSO or cytochalasin D+DMSO and 24 hours of culture in Example 7.

[FIG. 13] A diagram showing the Total Integrate Intensity (TII) representing phagocytic activity 24 hours after addition of lactic acid bacteria for the six species of lactic acid bacteria tested in Example 7.

[FIG. 14] A diagram showing the IFN-α concentration in the supernatant 24 hours after addition of lactic acid bacteria for the six species of lactic acid bacteria tested in Example 7.

Description of Embodiments

[0011] Embodiments for carrying out the present invention will be described below, but the present invention is not limited to the following embodiments.

[0012] The first embodiment of the present invention is an immunostimulatory composition comprising a bacterium as an active ingredient. In one aspect, the bacterium comprised as an active

ingredient in the immunostimulatory composition is a bacterium that is phagocytosed by a pDC. In one aspect, the bacterium comprised as an active ingredient in the immunostimulatory composition is a bacterium that binds to BDCA2.

[0013] Hereinafter, the "immunostimulatory composition comprising, as an active ingredient, a bacterium that is phagocytosed by a pDC" and/or the "immunostimulatory composition comprising, as an active ingredient, a bacterium that binds to BDCA2" may be referred to as the "immunostimulatory composition of the present embodiment."

[0014] The bacterium comprised in the immunostimulatory composition of the present embodiment (hereinafter, may be referred to as the "bacterium according to the present embodiment") may be a bacterium particularly useful for the human body. The bacterium according to the present embodiment may be a Gram-positive bacterium or a Gram-negative bacterium. For example, the bacterium according to the present embodiment may be a lactic acid bacterium or an acetic acid bacterium, and in a preferred aspect may be a lactic acid bacterium, and in a more preferred aspect may be a bacterium of the genus *Lactococcus*, and in a still more preferred aspect may be *Lactococcus lactis* subsp. *lactis*.

[0015] A lactic acid bacterium is a bacterium that produces lactic acid as a metabolite. Examples of lactic acid bacteria include bacteria of the genus *Oenococcus*, bacteria of the genus *Bifidobacterium*, bacteria of the genus *Weissella*, bacteria of the genus *Tetragenococcus*, bacteria of the genus *Lactococcus*, bacteria of the genus *Leuconostoc*, bacteria of the genus *Pediococcus*, bacteria of the genus *Streptococcus*, bacteria of the genus *Enterococcus*, bacteria of the genus *Lactobacillus*, acetic acid

bacteria, and bacteria of the genus Bacillus. In one aspect, the lactic acid bacterium may be a bacterium of the genus *Lactobacillus* or the genus *Lactococcus*.

[0016] Note that bacteria of the genus *Lactobacillus* in the present specification include bacteria that were classified in the genus *Lactobacillus* before the reclassification of the genus *Lactobacillus*. For example, bacteria newly classified into the genus *Acetilactobacillus*, the genus *Agrilactobacillus*, the genus *Amylolactobacillus*, the genus *Apilactobacillus*, the genus *Bombilactobacillus*, the genus *Companilactobacillus*, the genus *Dellaglioa*, the genus *Fructilactobacillus*, the genus *Furfurilactobacillus*, the genus *Holzapfelia*, the genus *Lacticaseibacillus*, the genus *Lactiplantibacillus*, the genus *Lapidilactobacillus*, the genus *Latilactobacillus*, the genus *Lentilactobacillus*, the genus *Levilactobacillus*, the genus *Ligilactobacillus*, the genus *Limosilactobacillus*, the genus *Liquorilactobacillus*, the genus *Loigolactobacillus*, the genus *Paralactobacillus*, the genus *Paucilactobacillus*, the genus *Schleiferilactobacillus*, the genus *Secundilactobacillus*, and the like in association with the reclassification of the genus *Lactobacillus* are included.

[0017] Among the above, preferred bacteria include bacteria of the genus *Oenococcus*, bacteria of the genus *Bifidobacterium*, bacteria of the genus *Lentilactobacillus*, bacteria of the genus *Weissella*, bacteria of the genus *Tetragenococcus*, bacteria of the genus *Lactococcus*, bacteria of the genus *Leuconostoc*, bacteria of the genus *Pediococcus*, bacteria of the genus *Enterococcus*, bacteria of the genus *Lactobacillus*, and

bacteria of the genus *Lactiplantibacillus*.

[0018] Examples of bacteria of the genus *Oenococcus* include *Oenococcus oeni* and the like. Specific examples of bacteria of the genus *Oenococcus* include *Oenococcus oeni* JCM 6125 and the like.

[0019] Examples of bacteria of the genus *Bifidobacterium* include *Bifidobacterium animalis* subsp. *lactis*, *Bifidobacterium longum* subsp. *infantis* and the like. Specific examples of bacteria of the genus *Bifidobacterium* include *Bifidobacterium animalis* subsp. *lactis* JCM 10602, *Bifidobacterium longum* subsp. *infantis* JCM 1222, *Bifidobacterium longum* subsp. *longum* BB536, *Bifidobacterium animalis* subsp. *lactis* BB-12, *Bifidobacterium longum* subsp. *infantis* M-63, *Bifidobacterium longum* subsp. *longum* N61, *Bifidobacterium bifidum* OLB6378, *Bifidobacterium breve* M-16V, *Bifidobacterium breve* MCC1274, *Bifidobacterium pseudolongum* JCM 1205, and the like.

[0020] Examples of bacteria of the genus *Weissella* include *Weissella paramesenteroides*, *Weissella viridescens* and the like. Specific examples of bacteria of the genus *Weissella* include *Weissella paramesenteroides* JCM 9890, *Weissella viridescens* JCM 1174, and the like.

[0021] Examples of bacteria of the genus *Tetragenococcus* include *Tetragenococcus halophilus* and the like. Specific examples of bacteria of the genus *Tetragenococcus* include *Tetragenococcus halophilus* NRIC 0098, *Tetragenococcus halophilus* No. 1, and the like.

[0022] Examples of bacteria of the genus *Lactococcus* include *Lactococcus lactis*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus*

garvieae, *Lactococcus lactis* subsp. cremoris, *Lactococcus lactis* subsp. hordniae, *Lactococcus plantarum*, and the like.

[0023] Specific examples of bacteria of the genus *Lactococcus* include *Lactococcus lactis* subsp. *lactis* JCM 5805, *Lactococcus lactis* subsp. *lactis* NBRC 12007, *Lactococcus lactis* subsp. *lactis* NRIC 1150, *Lactococcus lactis* subsp. *lactis* JCM 20101, *Lactococcus lactis* subsp. *lactis* JCM 7638, *Lactococcus lactis* subsp. *lactis* ATCC 7963, *Lactococcus lactis* subsp. *lactis* ATCC 7962, *Lactococcus lactis* subsp. *lactis* ATCC 29146, *Lactococcus lactis* subsp. *lactis* ATCC 27861, *Lactococcus lactis* subsp. *lactis* ATCC 19435, *Lactococcus lactis* subsp. *lactis* ATCC 15346, *Lactococcus lactis* subsp. *lactis* ATCC 13675, *Lactococcus lactis* subsp. *lactis* ATCC 12929, *Lactococcus lactis* subsp. *lactis* ATCC 11955, *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactococcus lactis* subsp. *lactis* ATCC 11007, *Lactococcus garvieae* NBRC 100934, *Lactococcus lactis* subsp. cremoris JCM 16167, *Lactococcus lactis* subsp. cremoris NBRC 100676, *Lactococcus lactis* subsp. hordniae JCM 1180, *Lactococcus lactis* subsp. hordniae JCM 11040, *Lactococcus plantarum* JCM 11056, and the like.

[0024] Examples of bacteria of the genus *Leuconostoc* include *Leuconostoc carnosum*, Leuconostoc lactis, and the like. Specific examples of bacteria of the genus *Leuconostoc* include *Leuconostoc carnosum* JCM 9695, Leuconostoc lactis NBRC 12455, and the like.

[0025] Examples of bacteria of the genus *Pediococcus* include *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Pediococcus cellicola*, *Pediococcus claussenii*, *Pediococcus damnosus*, *Pediococcus ethanolidurans*, *Pediococcus inopinatus*, *Pediococcus parvulus*,

Pediococcus stilesii, and the like. Specific examples of bacteria of the genus *Pediococcus* include, for example, *Pediococcus acidilactici* JCM 8797, *Pediococcus acidilactici* K15, *Pediococcus damnosus* JCM 5886, and the like.

[0026] Examples of bacteria of the genus *Streptococcus* include *Streptococcus thermophilus* and the like. Specific examples of bacteria of the genus *Pediococcus* include, for example, *Streptococcus thermophilus* SBC 8781 and the like.

[0027] Examples of bacteria of the genus *Enterococcus* include *Enterococcus alcedinis*, *Enterococcus faecalis* and the like. Specific examples of bacteria of the genus *Enterococcus* include *Enterococcus faecalis* EC-12 and the like.

[0028] Examples of bacteria of the genus *Lactobacillus* include *Lactobacillus paracasei*, *Lactobacillus delbrueckii*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus fructivorans*, *Lactobacillus hilgardii*, *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus parakefiri*, *Lactobacillus plantarum*, and *Lactobacillus pentosus*.

[0029] Specific examples of bacteria of the genus *Lactobacillus* include *Lactobacillus paracasei* KW3110, *Lactobacillus paracasei* MCC 1849, *Lactobacillus paracasei* K71, *Lactobacillus paracasei* K-2, *Lactobacillus rhamnosus* GG, *Lactobacillus rhamnosus* CRL1505, *Lactobacillus gasseri* SBT2055, *Lactobacillus gasseri* OLL2716, *Lactobacillus gasseri* PA-3, *Lactobacillus acidophilus* L-92, *Lactobacillus casei* subsp. *casei* 327, *Lactobacillus* (*Lacticaseibacillus* in the new classification) *casei Shirota*, *Lactobacillus bulgaricus*

OLL1073R-1, *Lactobacillus parakefiri* (*Lentilactobacillus parakefiri* in the new classification) JCM 8573, *Lactobacillus plantarum* (*Lactiplantibacillus plantarum* in the new classification) L-137, *Lactobacillus pentosus* (*Lactiplantibacillus pentosus* in the new classification) ONRICb0240, *Lactobacillus helveticus* GCL1815, and the like.

[0030] Acetic acid bacteria are not particularly limited, but examples include bacteria of the genus *Gluconacetobacter*, bacteria of the genus *Acetobacter*, bacteria of the genus *Gluconobacter*, and the like, with bacteria of the genus *Gluconacetobacter* being preferred, *Gluconacetobacter hansenii* being more preferred, and *Gluconacetobacter hansenii* GK-1 being still more preferred.

[0031] Bacteria of the genus *Bacillus* are not particularly limited, but examples include *Bacillus coagulans* and the like. Specific examples of bacteria of the genus *Bacillus* include, for example, *Bacillus coagulans* SANK70258 strain and the like.

[0032] Among the above, the bacterium according to the present embodiment is preferably selected from the group consisting of bacteria of the genus *Lactobacillus*, bacteria of the genus *Lactococcus*, and bacteria of the genus *Gluconacetobacter* from the viewpoint of flavor as a food.

[0033] In addition to the above, the bacterium according to the present embodiment may be a bacterium of the genus *Akkermansia*, a bacterium of the genus *Bacteroides*, a bacterium of the genus *Blautia*, a bacterium of the genus *Clostridium*, a bacterium of the genus *Collinsella*, a bacterium of the genus *Enterococcus*, a bacterium of the genus

Faecalibacterium, a bacterium of the genus *Faecalicatena*, a bacterium of the genus *Lacrimispora*, a bacterium of the genus *Paeniclostridium*, a bacterium of the genus *Parabacteroides*, or a bacterium of the genus *Roseburia*.

[0034] Examples of bacteria of the genus *Akkermansia* include *Akkermansia muciniphila* JCM 30893.

[0035] Examples of bacteria of the genus *Bacteroides* include *Bacteroides caccae* JCM 9498T, *Bacteroides fragilis* JCM 11019T, *Bacteroides fragilis* JCM 11017, *Bacteroides fragilis* JCM 17586, *Bacteroides fragilis* JCM 17587, *Bacteroides ovatus* JCM 5824T, *Bacteroides thetaiotaomicron* ATCC 29148T, *Bacteroides thetaiotaomicron* ATCC 29741, *Bacteroides thetaiotaomicron* ATCC 12290, *Bacteroides uniformis* JCM 5828T, *Bacteroides uniformis* JCM 13286, *Bacteroides uniformis* JCM 13287, and *Bacteroides uniformis* JCM 13288.

[0036] Examples of bacteria of the genus *Blautia* include *Blautia acetigignens* JCM 34803T, *Blautia ammoniilytica* JCM 34802T, *Blautia argi* JCM 31394T, *Blautia caecimuris* JCM 34498T, *Blautia coccoides* JCM 1395T, *Blautia faecis* JCM 17205T, *Blautia glucerasea* JCM 17039T, *Blautia hansenii* JCM 14655, *Blautia hansenii* JCM 35484, *Blautia hominis* JCM 32276T, *Blautia hydrogenotrophica* JCM 31266, *Blautia liquoris* JCM 34225T, *Blautia luti* JCM 17040T, *Blautia obeum* JCM 31340, *Blautia producta* JCM 1471T, *Blautia pseudococcoides* JCM 35243T, *Blautia schinkii* JCM 14657T, *Blautia wexlerae* JCM 31267, and *Blautia wexlerae* JCM 35486.

[0037] Examples of bacteria of the genus *Clostridium* include

Clostridium butyricum JCM NT, *Clostridium nexile* JCM 31500T, and *Clostridium symbiosum* JCM 1297T.

[0038] Examples of bacteria of the genus Collinsella include *Collinsella aerofaciens* JCM 10188T, *Collinsella intestinalis* JCM 10643T, *Collinsella stercoris* JCM 10641T, and *Collinsella tanakaei* JCM 16071T.

[0039] Examples of bacteria of the genus *Enterococcus* include *Enterococcus faecalis* JCM 5803T, *Enterococcus faecalis* JCM 20307, *Enterococcus faecium* JCM 5804T, and *Enterococcus faecium* JCM 8903.

[0040] Examples of bacteria of the genus *Faecalibacterium* include *Faecalibacterium hattorii* JCM 39210, *Faecalibacterium longum* JCM 39208, *Faecalibacterium prausnitzii* JCM 31915, *Faecalibacterium prausnitzii* JCM 39207, and *Faecalibacterium prausnitzii* JCM 39209.

[0041] Examples of bacteria of the genus *Faecalicatena* include *Faecalicatena oroticum* JCM 1429T.

[0042] Examples of bacteria of the genus *Lacrimispora* include *Lacrimispora celerecrescens* JCM 15734T, *Lacrimispora sphenoides* JCM 1415T, and *Lacrimispora xylanolytica* JCM 15735T.

[0043] Examples of bacteria of the genus Paeniclostridium include *Paeniclostridium sordellii* JCM 3814T.

[0044] Examples of bacteria of the genus *Parabacteroides* include Parabacteroides merdae JCM 9497T.

[0045] Examples of bacteria of the genus *Roseburia* include *Roseburia hominis* JCM 17582, *Roseburia intestinalis* JCM 17583, and *Roseburia inulinivorans* JCM 17584.

[0046] Among the above lactic acid bacteria and acetic acid bacteria, JCM strains can be obtained from the RIKEN BioResource Research Center, Microorganism Division (3-1-1 Koyadai, Tsukuba, Ibaraki), NBRC strains can be obtained from the Biological Resource Center, National Institute of Technology and Evaluation (2-5-8 Kazusakamatari, Kisarazu, Chiba), NRIC strains can be obtained from the Strain Preservation Laboratory, Tokyo University of Agriculture (1-1-1 Sakuragaoka, Setagaya, Tokyo), and ATCC strains can be obtained from the American Type Culture Collection (USA), respectively. In addition, the above lactic acid bacteria and acetic acid bacteria can be obtained not only from public institutions but also by isolating or purifying them from commercially available products containing lactic acid bacteria or acetic acid bacteria using known methods.

[0047] In addition, *Lactobacillus paracasei* KW3110 has been deposited as FERM BP-08634 at the International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology (1-1-1 Higashi, Tsukuba, Ibaraki, Japan, Central 6) (currently, the International Patent Organism Depositary, Biotechnology Center, National Institute of Technology and Evaluation (NITE-IPOD) (Room 120, 2-5-8 Kazusakamatari, Kisarazu, Chiba, Japan)), which is an international depositary authority under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure (date of deposit: February 20, 2004). In addition, a derivative strain of *Lactobacillus paracasei* KW3110 has been deposited as FERM BP-08635 at the same International Patent Organism Depositary (date of deposit: February 20, 2004).

[0048] *Lactococcus lactis* subsp. *lactis* JCM 5805 can be obtained from the RIKEN BioResource Research Center, Microorganism Division as described above, but in the present invention, the same strain of *Lactococcus lactis* subsp. *lactis* JCM 5805 stored at a depository institution other than the RIKEN BioResource Research Center, Microorganism Division can be used. Specifically, the same strain of *Lactococcus lactis* subsp. *lactis* JCM 5805 can be obtained from the Biological Resource Center, National Institute of Technology and Evaluation (2-5-8 Kazusakamatari, Kisarazu, Chiba, Japan), the Strain Preservation Laboratory, Tokyo University of Agriculture (1-1-1 Sakuragaoka, Setagaya, Tokyo, Japan), the American Type Culture Collection (USA), and the like. *Lactococcus lactis* subsp. *lactis* JCM 5805 has been deposited at the American Type Culture Collection as *Lactococcus lactis* subsp. *lactis* ATCC 9936 and *Lactococcus lactis* subsp. *lactis* ATCC 19435.

[0049] The bacterium according to the present embodiment may be a mutant strain of the strains included in the above enumeration. A mutant strain of a bacterium may be any strain as long as it has the properties capable of achieving the purpose of the present technology (for example, immunostimulatory ability in the case where the composition is an immunostimulatory composition). Such a mutant strain may be constructed by non-artificially introducing a mutation into the strains included in the above enumeration. Such a mutant strain may also be constructed by artificially introducing a mutation into the strains included in the above enumeration, for example, by introducing a mutation into the above bacterium by treatment with a mutagen such as

ultraviolet light (UV) or a DNA alkylating agent, or by introducing a mutation into the above strain by a gene manipulation method such as gene editing represented by CRISPR-Cas9.

[0050] The bacterium comprised in the immunostimulatory composition of the present embodiment may be a single species of bacterium or a mixture of two or more species of bacteria. The bacterium may be, for example, a mixture of a lactic acid bacterium and an acetic acid bacterium.

[0051] In one aspect, the bacterium according to the present embodiment is a bacterium that is phagocytosed by a pDC. That is, one aspect of the present embodiment is an immunostimulatory composition comprising, as an active ingredient, a bacterium that is phagocytosed by a pDC.

[0052] A plasmacytoid dendritic cell (pDC) is a type of dendritic cell constituting the innate immune system. pDCs are the major producing cells of type I interferon in the body. Type I interferons exhibit proliferation-inhibiting activity against viruses and the like. Interferon α (IFN-α) and interferon β (IFN-β) are known as representative type I interferons, and these are considered to have immunostimulatory ability.

[0053] Phagocytosis is a phenomenon that occurs when a cell takes in solid particles, other cells, bacteria, or other relatively large objects. In phagocytosis, the cell extends its cell membrane to engulf the object and takes it into the cell, after which a vesicle (phagosome) containing the ingested object fuses with a lysosome to degrade the contents.

[0054] It can be evaluated that a bacterium is a bacterium that is phagocytosed by a pDC, by an evaluation method using bacteria labeled

with a fluorogenic or fluorescent dye as described below. In addition, it can be evaluated that a bacterium is a bacterium that is phagocytosed by a pDC by a decrease in the uptake of the bacterium by the pDC when phagocytosis by the pDC is inhibited. For example, when the efficiency of uptake by the pDC in which phagocytosis is inhibited by an actin polymerization inhibitor (e.g., cytochalasin D, latrunculin B) is significantly lower than the efficiency of uptake by the pDC in which polymerization is not inhibited, the bacterium can be evaluated as a bacterium that is phagocytosed by the pDC. For example, when the efficiency of uptake by a pDC in which phagocytosis is inhibited by a ligand or antibody that binds to a protein on the surface of the pDC involved in phagocytosis of bacteria, such as BDCA2 described below, is significantly lower than the efficiency of uptake by the pDC in which binding to the protein on the surface of the pDC involved in phagocytosis of bacteria is not inhibited, the bacterium can be evaluated as a bacterium that is phagocytosed by the pDC. For the efficiency of uptake of bacteria by a pDC, for example, the amount of uptake at a predetermined time point after addition of bacteria to the pDC may be used as an indicator.

[0055] The bacterium of the present embodiment may be a bacterium in which the amount of phagocytosis by a pDC is 0.01 times or more, 0.1 times or more, 0.3 times or more, 0.5 times or more, 0.7 times or more, 0.9 times or more, or 1.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* JCM 5805 by the pDC. In addition, the bacterium according to the present embodiment may be a bacterium in which the amount of phagocytosis by a pDC is more than 1.0 times, 1.2 times or more, 1.5 times or more, 2.0 times or more, 3.0 times or more,

4.0 times or more, 5.0 times or more, 7.0 times or more, 10 times or more, 15 times or more, 20 times or more, 30 times or more, 40 times or more, 60 times or more, or 100 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC.

[0056] In addition, the amount of phagocytosis of the bacterium according to the present embodiment by a pDC can be evaluated by an evaluation method using bacteria labeled with a fluorogenic or fluorescent dye as described below. In the above evaluation method, for example, after bringing the bacterium according to the present embodiment and a control bacterium (*Lactococcus lactis* subsp. *lactis* JCM 5805, or *Lactococcus lactis* subsp. *lactis* ATCC 15577) into contact with a pDC of the same cell number (or same cell concentration) under the same conditions for a certain period of time, the bacteria are removed from the medium, and the fluorescence area per unit area of the observation field of a fluorescence microscope in the pDC (e.g., μm^2/image) can be evaluated as the amount of phagocytosis. In this case, the amount of phagocytosis is preferably the average value of multiple (2 or more, 3 or more, 5 or more, 7 or more, or 10 or more) fields of view or multiple (2 or more, 3 or more, 5 or more, 7 or more, or 10 or more) samples. When the amount of phagocytosis by a pDC is evaluated by an evaluation method using bacteria labeled with a fluorogenic or fluorescent dye as described below, the bacterium according to the present embodiment may have an amount of phagocytosis by the pDC of 50 μm^2/image or more, 100 μm^2/image or more, 200 μm^2/image or more, 400 μm^2/image or more, 500 μm^2/image or more, 600 μm^2/image or more, 700 μm^2/image or more, 800 μm^2/image or more, or 900

μm^2/image or more. Here, "image" means a field of view of 0.572 mm^2.

[0057] In addition, the difference obtained by subtracting the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by a pDC from the amount of phagocytosis of the bacterium according to the present embodiment by the pDC may be 1 μm^2/image or more, 20 μm^2/image or more, 50 μm^2/image or more, 70 μm^2/image or more, 100 μm^2/image or more, 140 μm^2/image or more, 200 μm^2/image or more, 300 μm^2/image or more, 400 μm^2/image or more, 500 μm^2/image or more, 600 μm^2/image or more, 700 μm^2/image or more, or 900 μm^2/image or more.

[0058] When the amount of phagocytosis by a pDC is evaluated using the Total Integrate Intensity (TII, OCU×μm^2/Image), which is the total amount of fluorescence per field of view of 0.572 mm^2, as an indicator by a measurement using the same protocol as the evaluation method using bacteria labeled with a fluorogenic or fluorescent dye as described below, the bacterium according to the present embodiment may have a TII of 10000 OCU×μm^2/Image or more, 30000 OCU×μm^2/Image or more, 60000 OCU×μm^2/Image or more, 100000 OCU×μm^2/Image or more, 150000 OCU×μm^2/Image or more, 200000 OCU×μm^2/Image or more, or 250000 OCU×μm^2/Image or more.

[0059] The amount of phagocytosis by a pDC in these cases is not particularly limited as long as it is a value of an indicator showing the amount of phagocytosis by the pDC, and the evaluation of the amount of phagocytosis by the pDC can be performed using methods commonly used by those skilled in the art as methods capable of measuring the amount of phagocytosis. For example, the amount of phagocytosis by a

pDC may be the fluorescence intensity of the pDC measured after contact with bacteria labeled with a fluorogenic or fluorescent dye. As a more detailed example, the amount of phagocytosis by a pDC can be evaluated using, as an indicator, the fluorescence detected from the pDC when bacteria labeled with a fluorescent dye that becomes strongly fluorescent in the acidic environment of endosomes (e.g., pHrodo Red SE, Invitrogen, P36600) are brought into contact with the pDC for a sufficient period of time (e.g., 24 hours). As indicators of fluorescence, those commonly used by those skilled in the art may be used, for example, the area or proportion thereof in a fluorescence image where the fluorescence intensity is greater than a predetermined value, the value obtained by multiplying the area where the fluorescence intensity is greater than a predetermined value by the average fluorescence intensity, the average value, median value, or histogram of fluorescence intensity in a fluorescence image, the histogram of fluorescence intensity by flow cytometry analysis or the proportion of cells included within a gate when gating is performed on fluorescence intensity, or the fluorescence intensity in bulk cells contained in a cell suspension or a microwell for microwell plate reader analysis and the like can be used. In addition, the amount of phagocytosis by a pDC may be evaluated using the expression level of a biomarker whose expression level varies according to the amount of phagocytosis by the pDC as an indicator. The values described above can be treated as the “amount of phagocytosis by a pDC” in the present embodiment.

[0060] In the present embodiment, the amount of phagocytosis by pDC may be the amount of phagocytosis evaluated using a CAL-1 cell. CAL-

1 cells are a cancer cell line of a human plasmacytoid dendritic cell (pDC) established from tumor cells in the peripheral blood of a patient, and are a cell line deposited at the Patent Microorganism Depositary, Biotechnology Center, National Institute of Technology and Evaluation (NPMD, Japan) under accession number FERM BP-10914. CAL-1 cells are disclosed in Japanese Unexamined Patent Application Publication No. 2007-044008 and the like.

[0061] The amount of phagocytosis by a pDC may be, as a detailed example, the amount of phagocytosis evaluated by the method described below as the "Evaluation method using bacteria labeled with a fluorogenic dye." The amount of phagocytosis by a pDC may be, as a more detailed example, the amount of phagocytosis evaluated by the method described in the Examples.

[0062] [Evaluation method using bacteria labeled with a fluorogenic dye]

(Phagocytosis experiment)

A cell suspension containing CAL-1 cells at 5.0×10^5 cells/mL is prepared using a serum-free medium (e.g., a medium prepared by adding penicillin/streptomycin (Gibco, 15140-12) at a final concentration of 1.0% by volume to RPMI-1640 medium (Sigma, R8758)), seeded at 10 mL/well in a 10 cm diameter culture dish, cultured for 16 hours, and collected by pipetting. The collected CAL-1 cells are resuspended in the same medium at a concentration of 2.0×10^5 cells/mL and seeded at 200 μL/well in each well of a 96-well microplate. Bacteria stained with pHrodo Red SE (Invitrogen, P36600) by the method described below are added at a final concentration of 10 μg/mL, and the fluorescence of

pHrodo Red SE in each well is measured over time using an Incucyte(registered trademark) SX5 Live-Cell Analysis System (Sartorius).

[0063] (Staining of bacteria with pHrodo Red SE)

pHrodo Red SE (Invitrogen, P36600) is dissolved in DMSO to prepare a 10.2 mM solution. The bacterial powder is weighed into a 2 mL Eppendorf tube, and 0.1 M sodium bicarbonate adjusted to pH 9.0 is added to prepare a bacterial solution of 20 mg/mL bacteria. 95 μL of the bacterial solution is transferred to a new 2 mL Eppendorf tube, and 5 μL of the 10.2 mM pHrodo Red SE prepared as described above is added. After dispersing the bacteria by vortexing, the tube is shielded from light with aluminum foil and incubated at room temperature for 60 minutes. Thereafter, 750 μL of PBS is added, and the mixture is vortexed and centrifuged at 20,000×g for 2 minutes at room temperature. After discarding 800 μL of the supernatant, 1.5 mL of PBS is added, and the pellet is completely resuspended by vortexing. After the mixture is centrifuged again at 20,000×g for 2 minutes at room temperature, 1.5 mL of the supernatant is discarded. 140 μL of PBS is added and the pellet is resuspended to prepare a bacterial suspension of 10 mg/mL stained bacteria, which is stored shielded from light with aluminum foil at 4°C until use. Immediately before use, the stained bacteria are diluted 10-fold with PBS and used as a bacterial suspension of 1 mg/mL bacteria. Note that the stained bacterial suspension is used within 24 hours of preparation.

[0064] (Evaluation of the amount of phagocytosis)

The amount of phagocytosis of bacteria by CAL-1 cells is quantified

using the total area in which fluorescence of pHrodo Red SE is detected (fluorescence detection area) in the entire field of view (0.572 mm^2) to be evaluated obtained from each well of the plate as an indicator. The fluorescence detection area per image (0.572 mm^2) (e.g., μm^2/image) can be analyzed according to the number of images to be evaluated using the Incucyte (registered trademark) SX5 Live-Cell Analysis System (Sartorius), and the amount of phagocytosis can be evaluated. At this time, since stained bacteria emit slight fluorescence even when not phagocytosed, the Threshold value of the Orange Channel is set to 5.0 to eliminate the influence of fluorescence not due to phagocytosis. In addition, since some types of bacteria may form aggregates and emit fluorescence exceeding the Threshold value even when not phagocytosed, the Area of Filters of the Orange Channel is set to 20 to eliminate the influence of excessively large aggregates.

[0065] The bacterium according to one aspect of the present embodiment that is phagocytosed by a pDC may satisfy the following Requirement X0. Note that the "ratio of the number of pDCs that have phagocytosed the bacterium to the total number of pDCs brought into contact" in the following corresponds to the "phagocytosis ratio" evaluated in Examples 8 and 9 described below. In one aspect, the pDC according to Requirement X0 may be a CAL-1 cell (FERM BP-10914).

[Requirement X0] After pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is a predetermined ratio,

provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[0066] The predetermined ratio according to Requirement X0 may be, for example, 2.5% or more, 3.0% or more, 4.0% or more, 5.0% or more, 5.5% or more, 6.5% or more, 7.0% or more, 7.5% or more, 8.0% or more, 9.0% or more, 9.5% or more, 10.0% or more, 10.5% or more, 11.0% or more, 11.5% or more, 12.0% or more, 12.5% or more, 13.0% or more, 13.5% or more, 14.0% or more, 14.5% or more, 15.0% or more, 15.5% or more, 16.0% or more, 16.5% or more, 17.0% or more, or 17.5% or more, and may be less than 100%, 50.0% or less, 25.0% or less, or 18.0% or less. These lower limit values and upper limit values can each be combined arbitrarily, and for example, the predetermined ratio according to Requirement X0 may be 2.5% or more and less than 100%, 2.5% or more and 50.0% or less, 2.5% or more and 25.0% or less, 2.5% or more and 18.0% or less, 3.0% or more and less than 100%, 3.0% or more and 50.0% or less, 3.0% or more and 25.0% or less, 3.0% or more and 18.0% or less, 4.0% or more and less than 100%, 4.0% or more and 50.0% or less, 4.0% or more and 25.0% or less, 4.0% or more and 18.0% or less, 5.0% or more and less than 100%, 5.0% or more and 50.0% or less, 5.0% or more and 25.0% or less, 5.0% or more and 18.0% or less, 5.5% or more and less than 100%, 5.5% or more and 50.0% or less, 5.5% or more and 25.0% or less, 5.5% or more and 18.0% or less, 6.5% or more and less than 100%, 6.5% or more and 50.0% or less, 6.5% or more and 25.0% or less, 6.5% or more and 18.0% or less, 7.0% or more and less than 100%, 7.0% or more and 50.0% or less, 7.0% or more and 25.0% or less, 7.0% or more and 18.0% or less, 7.5% or more and less than 100%, 7.5% or more and 50.0% or less, 7.5% or more and 25.0% or less,

7.5% or more and 18.0% or less, 8.0% or more and less than 100%, 8.0% or more and 50.0% or less, 8.0% or more and 25.0% or less, 8.0% or more and 18.0% or less, 9.0% or more and less than 100%, 9.0% or more and 50.0% or less, 9.0% or more and 25.0% or less, 9.0% or more and 18.0% or less, 9.5% or more and less than 100%, 9.5% or more and 50.0% or less, 9.5% or more and 25.0% or less, 9.5% or more and 18.0% or less, 10.0% or more and less than 100%, 10.0% or more and 50.0% or less, 10.0% or more and 25.0% or less, 10.0% or more and 18.0% or less, 10.5% or more and less than 100%, 10.5% or more and 50.0% or less, 10.5% or more and 25.0% or less, 10.5% or more and 18.0% or less, 11.0% or more and less than 100%, 11.0% or more and 50.0% or less, 11.0% or more and 25.0% or less, 11.0% or more and 18.0% or less, 11.5% or more and less than 100%, 11.5% or more and 50.0% or less, 11.5% or more and 25.0% or less, 11.5% or more and 18.0% or less, 12.0% or more and less than 100%, 12.0% or more and 50.0% or less, 12.0% or more and 25.0% or less, 12.0% or more and 18.0% or less, 12.5% or more and less than 100%, 12.5% or more and 50.0% or less, 12.5% or more and 25.0% or less, 12.5% or more and 18.0% or less, 13.0% or more and less than 100%, 13.0% or more and 50.0% or less, 13.0% or more and 25.0% or less, 13.0% or more and 18.0% or less, 13.5% or more and less than 100%, 13.5% or more and 50.0% or less, 13.5% or more and 25.0% or less, 13.5% or more and 18.0% or less, 14.0% or more and less than 100%, 14.0% or more and 50.0% or less, 14.0% or more and 25.0% or less, 14.0% or more and 18.0% or less, 14.5% or more and less than 100%, 14.5% or more and 50.0% or less, 14.5% or more and 25.0% or less, 14.5% or more and 18.0% or less,

15.0% or more and less than 100%, 15.0% or more and 50.0% or less, 15.0% or more and 25.0% or less, 15.0% or more and 18.0% or less, 15.5% or more and less than 100%, 15.5% or more and 50.0% or less, 15.5% or more and 25.0% or less, 15.5% or more and 18.0% or less, 16.0% or more and less than 100%, 16.0% or more and 50.0% or less, 16.0% or more and 25.0% or less, 16.0% or more and 18.0% or less, 16.5% or more and less than 100%, 16.5% or more and 50.0% or less, 16.5% or more and 25.0% or less, 16.5% or more and 18.0% or less, 17.0% or more and less than 100%, 17.0% or more and 50.0% or less, 17.0% or more and 25.0% or less, 17.0% or more and 18.0% or less, 17.5% or more and less than 100%, 17.5% or more and 50.0% or less, 17.5% or more and 25.0% or less, or 17.5% or more and 18.0% or less. When the predetermined ratio according to Requirement X0 is equal to or greater than the above lower limit values, the immunostimulatory ability of the composition tends to be higher compared to when the ratio is less than the above lower limit values.

[0067] Whether a certain bacterium satisfies Requirement X0 can be evaluated according to a method including pre-culturing a pDC in a serum-free medium (pre-treatment step), bringing the pDC obtained in the pre-treatment step into contact with the bacterium in a serum-free medium for 24 hours (pDC contact step), and evaluating the ratio of the number of pDCs that have phagocytosed the bacterium to the total number of pDCs brought into contact in the contact step (phagocytosis evaluation step).

[0068] The serum according to Requirement X0 generally refers to serum derived from humans or other animals used for culturing animal

cells including humans, and examples include human-derived serum, bovine-derived serum, and horse-derived serum, and more specifically, for example, fetal bovine serum (FBS) and the like.

[0069] The medium according to Requirement X0 may be the same medium as a medium commonly used by those skilled in the art for cell culture (e.g., a basal medium). For example, the medium may be a medium in which the solvent is water and which contains water, sugars such as D-glucose, and amino acids such as L-glutamine. The medium may further contain additives. The additives may be those commonly used by those skilled in the art for cell culture, and may be, for example, at least one selected from the group consisting of antibiotics (penicillin, streptomycin, etc.), vitamins, inorganic salts, and buffers. In one aspect, the D-glucose content of the medium can be 500 mg/L or more, or 1200 mg/L or more, and the D-glucose content of the medium can be 6000 mg/L or less, or 3000 mg/L or less. These upper limit values and lower limit values can each be combined arbitrarily, and for example, the D-glucose content of the medium may be 500 mg/L or more and 6000 mg/L or less, 500 mg/L or more and 3000 mg/L or less, 1200 mg/L or more and 6000 mg/L or less, or 1200 mg/L or more and 3000 mg/L or less. In one aspect, the L-glutamine content of the medium can be 100 mg/L or more, or 250 mg/L or more, and the L-glutamine content of the medium can be 1000 mg/L or less, or 600 mg/L or less. These upper limit values and lower limit values can each be combined arbitrarily, and for example, the L-glutamine content of the medium may be 100 mg/L or more and 1000 mg/L or less, 100 mg/L or more and 600 mg/L or less, 250 mg/L or more and 1000 mg/L or less, or 250 mg/L or more and 600

mg/L or less. The medium can be prepared by methods commonly performed by those skilled in the art. For example, it can be prepared by adding sugars, amino acids, and other components to water. For example, the medium can also be prepared by adding missing components to a basal medium that can be purchased or easily prepared by those skilled in the art, such as RPMI-1640 medium (Roswell Park Memorial Institute-1640 medium) and DMEM medium (Dulbecco's Modified Eagle Medium). The prepared medium may be sterilized using an autoclave or the like before use in the contact step. The medium according to a specific aspect may be RPMI-1640 medium.

[0070] The culture in the pre-treatment step may be performed under conditions commonly used by those skilled in the art for cell culture, and in a specific aspect may be performed in air at 37°C, 5% CO_2, and 100% humidity. Although a pDC is normally a suspension cell, a pDC exhibits an adherent cell-like appearance through the pre-treatment step, so the pre-treatment step is preferably performed using a culture vessel to which the pDC can adhere. Examples of culture vessels to which a pDC can adhere include cell-adhesive well plates (e.g., 96-well microplates) and glass-bottom dishes. The concentration of pDCs in the pre-treatment step is not particularly limited, and may be, for example, 0.1×10^6 cells/mL or more and 10.0×10^6 cells/mL or less as the concentration before the pDCs adhere, and in a specific aspect may be 5.0×10^5 cells/mL.

[0071] The culture time in the pre-treatment step can be, for example, 1 hour or more, 2 hours or more, 4 hours or more, 6 hours or more, or 8 hours or more, and can also be, for example, 72 hours or less, 48 hours

or less, 36 hours or less, 24 hours or less, 16 hours or less, 12 hours or less, 10 hours or less, or 8 hours or less. These upper limit values and lower limit values can each be combined arbitrarily, and in a specific aspect, the culture time in the pre-treatment step may be 16 hours.

[0072] As the medium in the pDC contact step, the same medium as described in the pre-treatment step can be used. The medium in the pDC contact step may be a medium of the same or different composition as the medium in the pre-treatment step, and in a specific aspect may be a medium of the same composition.

[0073] The ratio of the number of pDCs that have phagocytosed the bacterium to the total number of pDCs brought into contact is not particularly limited and can be evaluated according to methods that those skilled in the art may use for evaluating cell populations. Such evaluation methods may be, for example, a method of evaluating cells contained in a cell population per single cell at or a method of evaluating cells contained in a cell population in bulk, and in one aspect may be a method of evaluating cells contained in a cell population per single cell. In a method of evaluating cells contained in a cell population per single cell, for example, the ratio of the number of particles or solutions in which a signal derived from bacteria is also detected to the number of particles or solutions in which a signal derived from pDCs is detected can be evaluated as the ratio of the number of pDCs that have phagocytosed the bacterium to the total number of pDCs brought into contact. The signal derived from pDCs and the signal derived from bacteria may be, for example, scattered light or fluorescence. The signal derived from pDC may be, for example, scattered light, and in a specific aspect may be

forward scattered light (FSC) and side scattered light (SSC). The signal derived from bacteria may be, for example, a signal detected from bacteria or proteins contained therein, or a signal detected from nucleic acids amplified using DNA contained in bacteria as a template, and in one aspect may be, for example, a signal detected from bacteria or proteins contained therein.

[0074] In a preferred aspect, the method of evaluating cells contained in a cell population per single cell may be flow cytometry. That is, in a preferred aspect, the ratio of the number of pDCs that have phagocytosed the bacterium to the total number of pDCs brought into contact may be a ratio determined by flow cytometry.

[0075] In detail, the ratio of the number of pDCs that have phagocytosed the bacterium to the total number of pDCs brought into contact may be determined by flow cytometry, for example, by the method described below. Note that, in more detail, the ratio of the number of pDCs that have phagocytosed the bacterium to the total number of pDCs brought into contact may be determined, for example, according to the method carried out in Example 8 of the present application.

First, the bacteria used in the pDC contact step are fluorescently labeled by contacting a fluorescent dye with the bacteria or by genetic expression of a fluorescent protein for proteins contained therein.

Next, after the pDC contact step, dead cells contained in pDCs are fluorescently labeled by contacting them with a fluorescent dye, as necessary. The fluorescent dye for labeling dead cells is not particularly limited as long as it is one commonly used by those skilled in the art, and in a specific aspect may be 7-Amino-Actinomycin D (7-AAD). Note that

the fluorescent labeling of bacteria or proteins contained therein and the fluorescent labeling of dead cells are distinguishable from each other. Finally, the presence or absence of phagocytosis of bacteria by pDCs is evaluated using a flow cytometer. Measurement is performed according to conventional methods. In the evaluation of measurement results, although not particularly limited, for example, first, a cluster containing pDCs is selected using scattered light as an indicator, then, as necessary, a cluster containing living pDCs is selected from it as particles not labeled with dead cell labels, and finally, the proportion of particles in which fluorescence derived from the fluorescent dye labeling the bacteria is detected in that cluster is evaluated.

[0076] In a specific aspect, the composition according to the present embodiment may be a composition comprising *Lactococcus lactis* subsp. *lactis* JCM 5805 as a bacterium, wherein the *Lactococcus lactis* subsp. *lactis* JCM 5805 satisfies the following Requirement Y0. Note that the "ratio of the number of pDCs that have phagocytosed the bacterium to the total number of pDCs brought into contact" in the following corresponds to the " phagocytosis ratio" evaluated in Example 9 described below. In one aspect, the pDCs according to Requirement Y0 may be CAL-1 cells (FERM BP-10914). The composition according to the present aspect is not limited to immunostimulatory use, but the composition described for the present embodiment can be used, and the composition of the present aspect may be, for example, an immunostimulatory composition.

[Requirement Y0] After pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with *Lactococcus lactis* subsp. *lactis*

JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of pDCs that have phagocytosed *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[0077] The predetermined ratio according to Requirement Y0 may be, for example, 10.0% or more, 10.5% or more, 11.0% or more, 11.5% or more, 12.0% or more, 12.5% or more, 13.0% or more, 13.5% or more, 14.0% or more, 14.5% or more, 15.0% or more, 15.5% or more, 16.0% or more, 16.5% or more, 17.0% or more, or 17.5% or more, and may be less than 100%, 50.0% or less, 25.0% or less, or 18.0% or less. These lower limit values and upper limit values can each be combined arbitrarily, and for example, the predetermined ratio according to Requirement Y0 may be 10.0% or more and less than 100%, 10.0% or more and 50.0% or less, 10.0% or more and 25.0% or less, 10.0% or more and 18.0% or less, 10.5% or more and less than 100%, 10.5% or more and 50.0% or less, 10.5% or more and 25.0% or less, 10.5% or more and 18.0% or less, 11.0% or more and less than 100%, 11.0% or more and 50.0% or less, 11.0% or more and 25.0% or less, 11.0% or more and 18.0% or less, 11.5% or more and less than 100%, 11.5% or more and 50.0% or less, 11.5% or more and 25.0% or less, 11.5% or more and 18.0% or less, 12.0% or more and less than 100%, 12.0% or more and 50.0% or less, 12.0% or more and 25.0% or less, 12.0% or more and 18.0% or less, 12.5% or more and less than 100%, 12.5% or more and 50.0% or less, 12.5% or more and 25.0% or less, 12.5% or more and 18.0% or less, 13.0% or more and less than 100%, 13.0% or

more and 50.0% or less, 13.0% or more and 25.0% or less, 13.0% or more and 18.0% or less, 13.5% or more and less than 100%, 13.5% or more and 50.0% or less, 13.5% or more and 25.0% or less, 13.5% or more and 18.0% or less, 14.0% or more and less than 100%, 14.0% or more and 50.0% or less, 14.0% or more and 25.0% or less, 14.0% or more and 18.0% or less, 14.5% or more and less than 100%, 14.5% or more and 50.0% or less, 14.5% or more and 25.0% or less, 14.5% or more and 18.0% or less, 15.0% or more and less than 100%, 15.0% or more and 50.0% or less, 15.0% or more and 25.0% or less, 15.0% or more and 18.0% or less, 15.5% or more and less than 100%, 15.5% or more and 50.0% or less, 15.5% or more and 25.0% or less, 15.5% or more and 18.0% or less, 16.0% or more and less than 100%, 16.0% or more and 50.0% or less, 16.0% or more and 25.0% or less, 16.0% or more and 18.0% or less, 16.5% or more and less than 100%, 16.5% or more and 50.0% or less, 16.5% or more and 25.0% or less, 16.5% or more and 18.0% or less, 17.0% or more and less than 100%, 17.0% or more and 50.0% or less, 17.0% or more and 25.0% or less, 17.0% or more and 18.0% or less, 17.5% or more and less than 100%, 17.5% or more and 50.0% or less, 17.5% or more and 25.0% or less, or 17.5% or more and 18.0% or less. When the predetermined ratio according to Requirement Y0 is equal to or greater than the above lower limit values, the immunostimulatory ability of the composition tends to be higher compared to when the ratio is less than the above lower limit values. The predetermined ratio according to Requirement Y0 can be evaluated in accordance with the evaluation method for the predetermined ratio according to Requirement X0 described above.

[0078] *Lactococcus lactis* subsp. *lactis* JCM 5805 satisfying Requirement Y0 can be prepared, for example, according to the production method according to one aspect of the second embodiment described below.

[0079] The bacterium according to one aspect of the present embodiment has the property of binding to BDCA2 (blood dendritic cell antigen 2).

[0080] BDCA2 is a type of receptor protein and is expressed mainly on a plasmacytoid dendritic cell (pDC). In one aspect of the present embodiment, the bacterium according to the present embodiment may be a bacterium that selectively binds to BDCA2. In one aspect of the present embodiment, the bacterium according to the present embodiment may be a bacterium that specifically binds to BDCA2. That a bacterium binds to BDCA2 means that the BDCA2-binding ability is greater than the BDCA2-binding ability of *Lactococcus lactis* subsp. *lactis* ATCC 15577. That a bacterium selectively binds to BDCA2 means that the bacterium binds to BDCA2 with significant preference in a contaminated system containing BDCA2 (e.g., a system in which various proteins on the cell surface are present), for example, that the binding efficiency to a pDC expressing BDCA2 on the cell surface is statistically significantly greater (e.g., the p-value between the two groups is less than 0.05) than the binding efficiency to a pDC not expressing BDCA2 or a pDC in which BDCA2 on the cell surface has been blocked with an anti-BDCA2 antibody or the like. That a bacterium specifically binds to BDCA2 means that the bacterium binds only to BDCA2 in a contaminated system containing BDCA2 (e.g., a system in which various proteins on the cell

surface are present), except for non-specific adsorption, for example, that the binding efficiency to a pDC expressing BDCA2 on the cell surface is statistically remarkably greater (e.g., the p-value between the two groups is less than 0.01) than the binding efficiency to a pDC not expressing BDCA2 or a pDC in which BDCA2 on the cell surface has been blocked with an anti-BDCA2 antibody or the like.

[0081] It may be evaluated that a bacterium binds to BDCA2, by methods commonly performed by those skilled in the art, and may be evaluated, for example, by a binding assay. The binding assay may be an in vitro method capable of evaluating the binding of a bacterium to BDCA2 based on a predetermined detection indicator. The in vitro method may be an in vitro method not using cells (i.e., a cell-free in vitro method). The BDCA2 used in the binding assay may be BDCA2 existing in a free state in an aqueous medium, BDCA2 expressed in a cell line, or BDCA2 expressed in an artificial lipid bilayer membrane, and in a preferred aspect may be BDCA2 existing in a free state in an aqueous medium, and these BDCA2 may be purified. Such BDCA2 may be a commercially available product or may be one isolated and purified after being expressed in a host cell using a vector or the like. Such BDCA2 may be a chimeric protein to which an isolation tag such as a Flag tag is attached.

[0082] The bacterium according to the present embodiment may be a bacterium in which the binding ability to BDCA2 is 0.3 times or more, 0.5 times or more, 0.7 times or more, 0.9 times or more, or 1.0 times or more the binding ability of *Lactococcus lactis* subsp. *lactis* JCM 5805 to BDCA2. In addition, the bacterium according to the present embodiment

may be a bacterium in which the binding ability to BDCA2 is more than 1.0 times, 1.2 times or more, 1.5 times or more, 2.0 times or more, 2.5 times or more, 3.0 times or more, 5.0 times or more, 6.0 times or more, 10.0 times or more, 20.0 times or more, or 30.0 times or more the binding ability of *Lactococcus lactis* subsp. *lactis* ATCC 15577 to BDCA2.

[0083] The binding ability to BDCA2 in these cases is not particularly limited as long as it is a value of an indicator showing binding ability, and may be, for example, a value of an indicator commonly used by those skilled in the art for evaluating the binding ability of two substances, such as IC_{50}, or may be a value of an indicator showing the binding of a bacterium and BDCA2 evaluated in a binding assay according to one aspect described below. In a specific aspect, the binding ability to BDCA2 may be the proportion of bacteria bound to BDCA2 evaluated in a binding assay according to one aspect described below. When the indicator of binding ability to BDCA2 is the proportion of bacteria bound to BDCA2, for example, a bacterium in which 3% or more, 5% or more, 7% or more, 8% or more, 9% or more, 10% or more, 20% or more, 30% or more, 40% or more, 50% or more, 60% or more, 90% or more, or 95% or more of the cells on a cell number basis are bound to BDCA2 may be classified as a bacterium that binds to BDCA2.

[0084] In addition, when the binding ability to BDCA2 of the bacterium according to the present embodiment is evaluated as the proportion of bacteria bound to BDCA2 by a binding assay according to one aspect described below, the difference obtained by subtracting the BDCA2-binding ability of *Lactococcus lactis* subsp. *lactis* ATCC 15577 from the BDCA2-binding ability of the above bacterium may be 0.5% or more,

1.0% or more, 1.5% or more, 2.0% or more, 4.0% or more, 5.0% or more, 7.0% or more, 9.0% or more, 10.0% or more, 15.0% or more, 20.0% or more, 25.0% or more, 30.0% or more, or 40.0% or more.

[0085] One aspect of a binding assay for evaluating that a bacterium binds to BDCA2 is described below. One aspect of the binding assay includes a step of bringing BDCA2 into contact with a bacterium (contact step) and a step of evaluating the binding of BDCA2 to the bacterium after the contact step (evaluation step).

[0086] In the contact step, the bacterium and BDCA2 are brought into contact in an aqueous medium. The aqueous medium may be a buffer that those skilled in the art may use in in vitro methods using proteins, and may be, for example, PBS (phosphate-buffered saline), HBSS (Hanks' balanced salt solution), HEPES buffer, and Tris buffer, and media in which the salt concentration and pH thereof are appropriately adjusted. The aqueous medium may further contain additives that those skilled in the art may use in in vitro methods using proteins, such as surfactants, organic solvents (e.g., dimethyl sulfoxide and ethanol), and reducing agents (e.g., glutathione). The BDCA2 in the contact step may be BDCA2 existing in a free state in an aqueous medium.

[0087] The concentration of bacteria in the contact step is not particularly limited as long as the binding of BDCA2 to bacteria can be evaluated in the subsequent contact step. In one aspect, the concentration of bacteria in the contact step may be 0.01 μg/mL or more, 0.1 μg/mL or more, 1.0 μg/mL or more, or 3.0 μg/mL or more, and may be 10000 μg/mL or less, 1000 μg/mL or less, 100 μg/mL or less, or 30 μg/mL or less. In one aspect, these upper limit values and lower limit values can

each be combined arbitrarily, and for example, the concentration of bacteria in the contact step may be 0.01 μg/mL or more and 10000 μg/mL or less, 0.01 μg/mL or more and 1000 μg/mL or less, 0.01 μg/mL or more and 100 μg/mL or less, 0.01 μg/mL or more and 30 μg/mL or less, 0.1 μg/mL or more and 10000 μg/mL or less, 0.1 μg/mL or more and 1000 μg/mL or less, 0.1 μg/mL or more and 100 μg/mL or less, 0.1 μg/mL or more and 30 μg/mL or less, 1.0 μg/mL or more and 10000 μg/mL or less, 1.0 μg/mL or more and 1000 μg/mL or less, 1.0 μg/mL or more and 100 μg/mL or less, 1.0 μg/mL or more and 30 μg/mL or less, 3.0 μg/mL or more and 10000 μg/mL or less, 3.0 μg/mL or more and 1000 μg/mL or less, 3.0 μg/mL or more and 100 μg/mL or less, or 3.0 μg/mL or more and 30 μg/mL or less. In a specific aspect, the concentration of bacteria in the contact step may be 10 μg/mL.

[0088] The concentration of BDCA2 in the contact step is not particularly limited as long as the binding of BDCA2 to bacteria can be evaluated in the subsequent contact step. In one aspect, the concentration of BDCA2 may be 0.01 μg/mL or more, 0.1 μg/mL or more, or 0.3 μg/mL or more, and may be 5000 μg/mL or less, 2000 μg/mL or less, 1000 μg/mL or less, 500 μg/mL or less, or 200 μg/mL or less. In one aspect, these upper limit values and lower limit values can each be combined arbitrarily, and for example, the concentration of BDCA2 may be 0.01 μg/mL or more and 5000 μg/mL or less, 0.01 μg/mL or more and 2000 μg/mL or less, 0.01 μg/mL or more and 1000 μg/mL or less, 0.01 μg/mL or more and 500 μg/mL or less, 0.01 μg/mL or more and 200 μg/mL or less, 0.1 μg/mL or more and 5000 μg/mL or less, 0.1 μg/mL or more and 2000 μg/mL or less, 0.1 μg/mL or more and 1000 μg/mL or

less, 0.1 μg/mL or more and 500 μg/mL or less, 0.1 μg/mL or more and 200 μg/mL or less, 0.3 μg/mL or more and 5000 μg/mL or less, 0.3 μg/mL or more and 2000 μg/mL or less, 0.3 μg/mL or more and 1000 μg/mL or less, 0.3 μg/mL or more and 500 μg/mL or less, or 0.3 μg/mL or more and 200 μg/mL or less. In a specific aspect, the concentration of bacteria in the contact step may be 10 μg/mL.

[0089] The concentration ratio of bacteria to BDCA2 in the contact step is not particularly limited as long as the binding of BDCA2 to bacteria can be evaluated in the subsequent contact step. In one aspect, the concentration of BDCA2 [μg/mL] in the contact step may be 0.01 times or more, 0.03 times or more, 0.1 times or more, or 0.3 times or more the concentration of bacteria [μg/mL], and may be 100 times or less, 30 times or less, 10 times or less, or 3 times or less. In one aspect, these upper limit values and lower limit values can each be combined arbitrarily, and for example, the concentration of BDCA2 [μg/mL] in the contact step may be 0.01 times or more and 100 times or less, 0.01 times or more and 30 times or less, 0.01 times or more and 10 times or less, 0.01 times or more and 3 times or less, 0.03 times or more and 100 times or less, 0.03 times or more and 30 times or less, 0.03 times or more and 10 times or less, 0.03 times or more and 3 times or less, 0.1 times or more and 100 times or less, 0.1 times or more and 30 times or less, 0.1 times or more and 10 times or less, 0.1 times or more and 3 times or less, 0.3 times or more and 100 times or less, 0.3 times or more and 30 times or less, 0.3 times or more and 10 times or less, or 0.3 times or more and 3 times or less the concentration of bacteria [μg/mL]. In a specific aspect, the concentration of BDCA2 [μg/mL] in the contact step may be 1.0

times the concentration of bacteria [μg/mL].

[0090] Contact conditions such as time and temperature in the contact step can be appropriately determined by those skilled in the art so that the binding of BDCA2 to bacteria can be evaluated in the subsequent contact step. For example, the contact time in the contact step is not particularly limited as long as the binding of BDCA2 to bacteria can be evaluated in the subsequent contact step. In one aspect, the contact time in the contact step may be 1 minute or more, 10 minutes or more, or 30 minutes or more, and may be 24 hours or less, 6 hours or less, or 2 hours or less. In one aspect, these upper limit values and lower limit values can each be combined arbitrarily, and for example, the contact time in the contact step may be 1 minute or more and 24 hours or less, 1 minute or more and 6 hours or less, 1 minute or more and 2 hours or less, 10 minutes or more and 24 hours or less, 10 minutes or more and 6 hours or less, 10 minutes or more and 2 hours or less, 30 minutes or more and 24 hours or less, 30 minutes or more and 6 hours or less, or 30 minutes or more and 2 hours or less. In a specific aspect, the contact time in the contact step may be 1 hour. A more specific example of the contact step includes a step in accordance with the BDCA2 binding assay described in the Examples of the present specification.

[0091] In the evaluation step, the binding of BDCA2 to bacteria after the contact step is evaluated. The evaluation method for binding in the evaluation step is not particularly limited as long as it can evaluate the binding of BDCA2 to bacteria, and may be, for example, flow cytometry, ELISA, radioimmunoassay, surface plasmon resonance, or isothermal titration calorimetry. In a specific aspect, the evaluation method for

binding in the evaluation step may be flow cytometry. When evaluating the binding of BDCA2 to bacteria by flow cytometry, for example, by labeling BDCA2 and bacteria with fluorescent dyes of different colors, the binding of BDCA2 to bacteria can be evaluated based on the fluorescence intensity at wavelengths corresponding to those fluorescent dyes. BDCA2 and bacteria may be labeled before the contact step, or may be labeled after the contact step and before the evaluation step by a BDCA2-selective or bacteria-selective labeling method (e.g., labeling using a protein tag, labeling using a specific antibody, labeling using a secondary antibody selective for the origin of bacteria).

[0092] The indicator of binding of BDCA2 to bacteria evaluated in the evaluation step is not particularly limited as long as it is a value of an indicator showing binding ability, and may be, for example, a value of an indicator commonly used by those skilled in the art for evaluating the binding ability of two substances, such as IC_{50}, or may be a value of an indicator showing the binding ability of each bacterium to BDCA2 (e.g., the proportion of bacteria bound to BDCA2, or the average or median number of BDCA2 labels per bacterium). In a specific aspect, the indicator of binding of BDCA2 to bacteria evaluated in the evaluation step may be the proportion of bacteria bound to BDCA2. The proportion of bacteria bound to BDCA2 can be calculated, for example, as the proportion of particles in which fluorescence intensity at a wavelength corresponding to the fluorescent dye labeling BDCA2 is observed at a certain level or more among particles in which fluorescence at a wavelength corresponding to the fluorescent dye labeling bacteria is observed. As the criterion for a certain level or more, for example, when

using a chimeric protein of BDCA2 with an Fc region bound thereto, the threshold can be set so that the proportion of bacteria to which the control having only the Fc region binds is 0.1% or less.

[0093] In this way, according to one aspect of the binding assay, the binding ability of bacteria to BDCA2 can be evaluated. In addition, the present inventors found that bacteria that bind to BDCA2 have immunostimulatory ability. Then, using binding to BDCA2 as an indicator, the immunostimulatory ability of bacteria can be evaluated and bacteria having immunostimulatory ability can be screened, and such indicators can be obtained by a binding assay. That is, one embodiment of the present invention is a method for screening a bacterium having immunostimulatory ability, comprising a step of screening using binding to BDCA2 as an indicator, and in one aspect, the screening step is performed by a binding assay between a candidate bacterium and BDCA2. The candidate bacterium may be the same as the bacterium of one embodiment of the present invention. The binding assay is as described above.

[0094] The bacterium according to the present embodiment (i.e., a bacterium that is phagocytosed by a pDC and/or a bacterium that binds to BDCA2) may be a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) via binding to BDCA2.

[0095] The bacterium according to the present embodiment may be a bacterium in which the amount of phagocytosis via binding to BDCA2 by a pDC is 0.1 times or more, 0.3 times or more, 0.5 times or more, 0.7 times or more, 0.9 times or more, or 1.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* JCM 5805 by the pDC

via binding to BDCA2. In addition, the bacterium according to the present embodiment may be a bacterium in which the amount of phagocytosis via binding to BDCA2 by a pDC is more than 1.0 times, 1.2 times or more, 1.5 times or more, 2.0 times or more, 3.0 times or more, 4.0 times or more, 5.0 times or more, 7.0 times or more, 10 times or more, 20 times or more, 30 times or more, 40 times or more, 60 times or more, or 100 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC via binding to BDCA2.

[0096] In addition, when the amount of phagocytosis via binding to BDCA2 by a pDC is evaluated by the evaluation method described below, the bacterium according to the present embodiment may have an amount of phagocytosis via binding to BDCA2 by a pDC of 10 μm^2/image or more, 20 μm^2/image or more, 30 μm^2/image or more, 50 μm^2/image or more, 100 μm^2/image or more, 200 μm^2/image or more, 400 μm^2/image or more, 500 μm^2/image or more, 600 μm^2/image or more, 700 μm^2/image or more, 800 μm^2/image or more, or 900 μm^2/image or more. Note that, as described in the above (Evaluation of phagocytosis amount), the area per image is set to 0.572 mm^2.

[0097] In addition, the difference obtained by subtracting the amount of phagocytosis via binding to BDCA2 by a pDC of *Lactococcus lactis* subsp. *lactis* ATCC 15577 from the amount of phagocytosis via binding to BDCA2 by the pDC of the above bacterium may be 1 μm^2/image or more, 20 μm^2/image or more, 40 μm^2/image or more, 70 μm^2/image or more, 100 μm^2/image or more, 140 μm^2/image or more, 200 μm^2/image or more, 300 μm^2/image or more, 400 μm^2/image or more, 500

μm^2/image or more, 600 μm^2/image or more, 700 μm^2/image or more, or 900 μm^2/image or more. Note that, as described in the above (Evaluation of phagocytosis amount), the area per image is set to 0.572 mm^2.

[0098] In one aspect, the phagocytosis of bacteria by a pDC includes phagocytosis via binding to BDCA2 and phagocytosis not via binding to BDCA2, and the amount of phagocytosis via binding to BDCA2 may be a predetermined multiple or more of the amount of phagocytosis not via binding to BDCA2. The predetermined multiple may be, for example, 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, or 10 times. In addition, in the phagocytosis of the bacterium by a pDC, the amount of phagocytosis via binding to BDCA2 among the total amount of phagocytosis may be, for example, 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, or 90% or more. A bacterium in which the amount of phagocytosis via binding to BDCA2 is a predetermined multiple or more of the amount of phagocytosis not via binding to BDCA2 is easily phagocytosed by a pDC via binding to BDCA2, and therefore has high pDC-activating ability and high immunostimulatory ability.

[0099] The amount of phagocytosis via binding to BDCA2 by a pDC in these cases is not particularly limited as long as it is a value of an indicator showing the amount of phagocytosis via binding to BDCA2 by the pDC. For example, the amount of phagocytosis via binding to BDCA2 and the amount of phagocytosis not via binding to BDCA2 in the phagocytosis of bacteria by a pDC can be evaluated as follows. First, the amount of phagocytosis of bacteria by untreated a pDC

(phagocytosis amount P) is evaluated. Next, the amount of phagocytosis of bacteria by a pDC subjected to a treatment that suppresses BDCA2 in a state capable of binding to a ligand on the cell surface (phagocytosis amount Q) is evaluated. Then, phagocytosis amount Q represents the amount of phagocytosis not via binding to BDCA2 by a pDC. In addition, the value obtained by subtracting phagocytosis amount Q from phagocytosis amount P represents the amount of phagocytosis via binding to BDCA2 by a pDC. That is, in one aspect, the amount of phagocytosis not via binding to BDCA2 may be the amount of phagocytosis of the bacterium by a pDC in a state where BDCA2 of a pDC cannot bind to a ligand.

[0100] The treatment that suppresses BDCA2 in a state capable of binding to a ligand may be, for example, exposing a pDC to an anti-BDCA2 antibody (blocking), exposing a pDC to a ligand of BDCA (competitive inhibition), knocking down or knocking out BDCA2 of a pDC according to conventional methods, or introducing a mutation into BDCA2 of a pDC according to conventional methods to abolish the binding ability to a ligand. In a preferred aspect, the treatment that suppresses BDCA2 in a state capable of binding to a ligand may be exposing a pDC to an anti-BDCA2 antibody. In this case, the state in which BDCA2 on the cell surface of pDCs is bound to an anti-BDCA2 antibody corresponds to the state in which BDCA2 of a pDC cannot bind to a ligand. In these cases, the anti-BDCA2 antibody may be a monoclonal antibody or a polyclonal antibody, and in a preferred aspect may be a monoclonal antibody. For example, the anti-BDCA2 antibody can be obtained from suppliers such as Miltenyi Biotec (catalog number:

130-090-690).

[0101] The amount of phagocytosis via binding to BDCA2 by a pDC may be, as a detailed example, the amount of phagocytosis evaluated by the method described below as the "Evaluation method for the amount of phagocytosis via binding to BDCA2 and the amount of phagocytosis not via binding to BDCA2." The amount of phagocytosis via binding to BDCA2 by a pDC may be, as a more detailed example, the amount of phagocytosis evaluated by the method described in the Examples.

[Evaluation method for the amount of phagocytosis via binding to BDCA2 and the amount of phagocytosis not via binding to BDCA2]

To CAL-1 cell suspension in each well seeded in the same manner as in the phagocytosis experiment of the above-described [Evaluation method using bacteria labeled with a fluorogenic dye], bacteria and an anti-BDCA2 antibody (Miltenyi Biotec, 130-090-690), or bacteria and an isotype control of the same isotype and subclass as the anti-BDCA2 antibody (Miltenyi Biotec, 130-106-545) are added so that both are at 10 μg/mL, and incubated for 30 minutes. Thereafter, for each, the fluorescence intensity of pHrodo Red SE is measured in the same manner as in the evaluation of phagocytosis amount in the above-described [Evaluation method using bacteria labeled with a fluorogenic dye], and a fluorescence detection area per image (e.g., μm^2/image) is obtained. Then, the fluorescence detection area when the anti-BDCA2 antibody is added is taken as the above-described phagocytosis amount Q, and the fluorescence detection area when the isotype control is added is taken as the above-described phagocytosis amount P, and the amount of phagocytosis via binding to BDCA2 (P-Q) and the amount of

phagocytosis not via binding to BDCA2 (P) are evaluated.

[0102] In the immunostimulatory composition of the present embodiment, the bacterium may be a live bacterium or a dead bacterium, or may be a mixture of a live bacterium and a dead bacterium. The bacterium according to the immunostimulatory composition of the present embodiment may be composed of dead bacteria.

[0103] The bacterium comprised in the immunostimulatory composition of the present embodiment has high immunostimulatory ability. The immunostimulatory ability of the bacterium comprised in the immunostimulatory composition of the present embodiment may be 0.01 times or more, 0.03 times or more, 0.05 times or more, 0.07 times or more, 0.09 times or more, 0.1 times or more, 0.3 times or more, 0.5 times or more, 0.7 times or more, 0.9 times or more, or 1.0 times or more the pDC immunostimulatory ability of *Lactococcus lactis* subsp. *lactis* JCM 5805. In addition, the immunostimulatory ability of the bacterium comprised in the immunostimulatory composition of the present embodiment may be more than 1.0 times, 1.2 times or more, 1.5 times or more, 2.0 times or more, 3.0 times or more, 4.0 times or more, 5.0 times or more, 7.0 times or more, 10 times or more, 20 times or more, 30 times or more, 40 times or more, 60 times or more, or 100 times or more the immunostimulatory ability of *Lactococcus lactis* subsp. *lactis* ATCC 15577. The immunostimulatory ability in these cases is not particularly limited as long as it is a parameter that those skilled in the art may use as an indicator of immunostimulatory ability, but in a preferred aspect may be the expression level of IFN-α.

[0104] The immunostimulatory ability (immune-activating ability) in

the present specification means the ability to stimulate cellular immunity and/or humoral immunity, and is the stimulating (activating) action on the innate immune system in cells contacted with or animals administered with the bacterium according to the present embodiment. In one aspect, the immunostimulatory ability may be a dendritic cell-activating ability that activates the innate immune system of dendritic cells, and more specifically may be a plasmacytoid dendritic cell-activating ability (pDC-activating ability). In one aspect, the immunostimulatory ability may be an increase in the expression level of cytokines secreted from dendritic cells or cell surface markers, preferably an increase in the expression level of IFN-α, and may be an increase in the expression level of IFN-α production by a pDC. In vivo, a pDC is the main producer of IFN-α, and IFN-α is a type of cytokine responsible for the biological defense mechanism by the innate immune system. Therefore, a substance having the ability to promote IFN-α expression by a pDC can also be regarded as a substance having dendritic cell-activating ability and pDC-activating ability, and can also be regarded as a substance having immunostimulatory ability.

[0105] For example, the immunostimulatory ability of a bacterium can be evaluated using the expression level of IFN-α by a pDC after contact with the bacterium as an indicator. The expression level of IFN-α by a pDC is the amount of a biomolecule that can serve as an evaluation indicator for the amount of IFN-α expressed by the pDC due to bacteria. In one aspect, the expression level of IFN-α may be the amount of IFN-α secreted extracellularly and/or the amount of IFN-α in a pDC, or the amount of mRNA encoding IFN-α in a pDC. In a preferred aspect, the

expression level of IFN-α may be the amount of IFN-α secreted extracellularly and adsorbed to cell culture wells measured by the ELISPOT method, or the amount of IFN-α secreted extracellularly and present in the culture supernatant. The culture supernatant in these cases is the medium after bringing bacteria into contact with a pDC in the contact step. The amount of IFN-α in the culture supernatant can be measured after collecting the culture supernatant according to methods commonly used by those skilled in the art for quantifying specific proteins. In addition, the amount of IFN-α in a pDC (e.g., in a CAL-1 cell) can be measured by methods commonly performed by those skilled in the art, for example, by intracellular cytokine staining (Intracellular Cytokine Staining) using a flow cytometer, or by preparing a lysate of pDCs and then measuring the amount of IFN-α in the lysate according to methods commonly used by those skilled in the art for quantifying specific proteins. The measurement of the amount of IFN-α in these cases may be performed by, for example, ELISA or Western blotting, and may be performed by ELISA. For the measurement of IFN-α amount by ELISA, for example, a Human IFN-Alpha Multi-Subtype ELISA Kit (PBL Assay Science) can be used. In addition, the amount of mRNA encoding IFN-α in a pDC (e.g., in CAL-1 cells) can be measured by preparing a lysate of pDCs by methods commonly performed by those skilled in the art and then measuring the amount of mRNA encoding IFN-α in the lysate according to methods commonly used by those skilled in the art for quantifying mRNA. The quantification of mRNA may be performed by, for example, quantitative PCR methods (q-PCR methods) such as real-time PCR, direct digital counting (e.g., nCounter

(registered trademark)), or methods using next-generation sequencers (NGS).

[0106] When the immunostimulatory ability of a bacterium is evaluated using the expression level of IFN-α by a pDC after contact with the bacterium as an indicator, the indicator may be the expression level of IFN-α at a predetermined time (e.g., 3 to 168 hours later, for example 22 hours later) after bringing the bacterium into contact with a pDC (e.g., a CAL-1 cell) in a serum-free medium (e.g., a medium prepared by adding penicillin/streptomycin (Gibco, 15140-12) at a final concentration of 1.0% by volume to RPMI-1640 medium (Sigma, R8758)), and preferably may be the amount of IFN-α secreted extracellularly and present in the culture supernatant after treatment under the same conditions. As a more detailed example, the indicator of immunostimulatory ability of bacteria may be the amount of IFN-α in the culture supernatant processed and measured by the same method as in the Examples.

[0107] In one aspect where the bacterium according to the present embodiment is a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) via binding to BDCA2, the bacterium according to the present embodiment may promote production of IFN-α in a pDC via phagocytosis by the pDC. That is, the bacterium according to the present embodiment may promote production of IFN-α in a pDC via phagocytosis by the pDC subsequent to binding to BDCA2.

[0108] It can be determined that a bacterium promotes production of IFN-α in a pDC via phagocytosis by the pDC subsequent to binding to BDCA2, by the fact that the amount of IFN-α production by a pDC

brought into contact with BDCA2 after being subjected to a treatment that suppresses BDCA2 in a state capable of binding to a ligand is smaller than the amount of IFN-α production by a pDC not subjected to such treatment. The bacterium according to the present embodiment may be a bacterium in which the amount of IFN-α production by a pDC brought into contact with BDCA2 after being subjected to a treatment that suppresses BDCA2 in a state capable of binding to a ligand is 1/2 times or less, 1/3 times or less, 1/4 times or less, 1/5 times or less, 1/6 times or less, 1/7 times or less, 1/8 times or less, 1/9 times or less, or 1/10 times or less the amount of IFN-α production by a pDC not subjected to such treatment. In these cases, the treatment that suppresses BDCA2 in a state capable of binding to a ligand and the amount of IFN-α production are as already described above.

[0109] That a bacterium promotes production of IFN-α in a pDC via phagocytosis by the pDC subsequent to binding to BDCA2 may be that the ratio of the fold change of the amount of IFN-α production to the fold change of the amount of phagocytosis via BDCA2 in the pDC is more than 0 times, 0.1 times or more, 0.2 times or more, 0.3 times or more, 0.4 times or more, 0.5 times or more, 0.6 times or more, 0.7 times or more, 0.8 times or more, 0.9 times or more, 1.0 times or more, 1.2 times or more, 1.4 times or more, 1.6 times or more, 1.8 times or more, or 2.0 times or more. In the above case, that the ratio of the fold change of the amount of IFN-α production to the fold change of the amount of phagocytosis via BDCA2 in a pDC is X times or more means that when the amount of phagocytosis via BDCA2 in the pDC increases by Y%, the amount of IFN-α production in the pDC increases by X×Y% or more,

and/or when the amount of phagocytosis via BDCA2 in a pDC decreases by Z%, the amount of IFN-α production in the pDC decreases by X×Z% or more.

[0110] The immunostimulatory composition of the present embodiment may be a food composition, a pharmaceutical composition, a quasi-drug, a bacterial powder (a powder obtained by drying bacterial cells or a powder containing the same, etc.), an additive, or a feed, and may preferably be a food composition. The immunostimulatory ability (immune-activating ability) is the stimulating (activating) action on the innate immune system on cells or living organisms. The immunostimulatory composition according to one aspect may be a composition for activating dendritic cell, and may be a composition for activating plasmacytoid dendritic cell (for activating a pDC). The immunostimulatory composition of the present embodiment can be implemented in accordance with the following description.

[0111] The content of the bacterium according to the present embodiment is not particularly limited and as long as it is an amount satisfying an effective amount for immunostimulation, and therefore may vary depending on the form of the immunostimulatory composition of the present embodiment. For example, the dry weight of the bacterium according to the present embodiment relative to the total dry weight of the immunostimulatory composition of the present embodiment may be 0.0001 mass% or more, 0.001 mass% or more, 0.005 mass% or more, 0.01 mass% or more, 0.02 mass% or more, 0.05 mass% or more, 0.10 mass% or more, 0.30 mass% or more, 1.0 mass% or more, 1.5 mass% or more, 2.0 mass% or more, 2.5 mass% or more, 3.0 mass% or more, 5.0

mass% or more, 7.0 mass% or more, 10.0 mass% or more, 80.0 mass% or more, 90.0 mass% or more, 95.0 mass% or more, 99.0 mass% or more, or 100 mass%, and may be 100 mass% or less, 95 mass% or less, 90 mass% or less, 80 mass% or less, 70 mass% or less, 60 mass% or less, 50 mass% or less, 30 mass% or less, 25 mass% or less, 20 mass% or less, or 15 mass% or less. These lower limit values and upper limit values can each be combined arbitrarily, and for example, the dry weight of the bacterium relative to the total dry weight of the composition of the present embodiment may be 0.0001 mass% or more and 100 mass% or less, 0.0001 mass% or more and 95 mass% or less, 0.0001 mass% or more and 90 mass% or less, 0.0001 mass% or more and 80 mass% or less, 0.0001 mass% or more and 70 mass% or less, 0.0001 mass% or more and 60 mass% or less, 0.0001 mass% or more and 50 mass% or less, 0.0001 mass% or more and 30 mass% or less, 0.0001 mass% or more and 25 mass% or less, 0.0001 mass% or more and 20 mass% or less, 0.0001 mass% or more and 15 mass% or less, 0.0001 mass% or more and 10 mass% or less, 0.001 mass% or more and 100 mass% or less, 0.001 mass% or more and 95 mass% or less, 0.001 mass% or more and 90 mass% or less, 0.001 mass% or more and 80 mass% or less, 0.001 mass% or more and 70 mass% or less, 0.001 mass% or more and 60 mass% or less, 0.001 mass% or more and 50 mass% or less, 0.001 mass% or more and 30 mass% or less, 0.001 mass% or more and 25 mass% or less, 0.001 mass% or more and 20 mass% or less, 0.001 mass% or more and 15 mass% or less, 0.001 mass% or more and 10 mass% or less, 0.005 mass% or more and 100 mass% or less, 0.005 mass% or more and 95 mass% or less, 0.005 mass% or more and 90

mass% or less, 0.005 mass% or more and 80 mass% or less, 0.005 mass% or more and 70 mass% or less, 0.005 mass% or more and 60 mass% or less, 0.005 mass% or more and 50 mass% or less, 0.005 mass% or more and 30 mass% or less, 0.005 mass% or more and 25 mass% or less, 0.005 mass% or more and 20 mass% or less, 0.005 mass% or more and 15 mass% or less, 0.005 mass% or more and 10 mass% or less, 0.01 mass% or more and 100 mass% or less, 0.01 mass% or more and 95 mass% or less, 0.01 mass% or more and 90 mass% or less, 0.01 mass% or more and 80 mass% or less, 0.01 mass% or more and 70 mass% or less, 0.01 mass% or more and 60 mass% or less, 0.01 mass% or more and 50 mass% or less, 0.01 mass% or more and 30 mass% or less, 0.01 mass% or more and 25 mass% or less, 0.01 mass% or more and 20 mass% or less, 0.01 mass% or more and 15 mass% or less, 0.01 mass% or more and 10 mass% or less, 0.02 mass% or more and 100 mass% or less, 0.02 mass% or more and 95 mass% or less, 0.02 mass% or more and 90 mass% or less, 0.02 mass% or more and 80 mass% or less, 0.02 mass% or more and 70 mass% or less, 0.02 mass% or more and 60 mass% or less, 0.02 mass% or more and 50 mass% or less, 0.02 mass% or more and 30 mass% or less, 0.02 mass% or more and 25 mass% or less, 0.02 mass% or more and 20 mass% or less, 0.02 mass% or more and 15 mass% or less, 0.02 mass% or more and 10 mass% or less, 0.05 mass% or more and 100 mass% or less, 0.05 mass% or more and 95 mass% or less, 0.05 mass% or more and 90 mass% or less, 0.05 mass% or more and 80 mass% or less, 0.05 mass% or more and 70 mass% or less, 0.05 mass% or more and 60 mass% or less, 0.05 mass% or more and 50 mass% or less, 0.05 mass% or more and 30

mass% or less, 0.05 mass% or more and 25 mass% or less, 0.05 mass% or more and 20 mass% or less, 0.05 mass% or more and 15 mass% or less, 0.05 mass% or more and 10 mass% or less, 0.10 mass% or more and 100 mass% or less, 0.10 mass% or more and 95 mass% or less, 0.10 mass% or more and 90 mass% or less, 0.10 mass% or more and 80 mass% or less, 0.10 mass% or more and 70 mass% or less, 0.10 mass% or more and 60 mass% or less, 0.10 mass% or more and 50 mass% or less, 0.10 mass% or more and 30 mass% or less, 0.10 mass% or more and 25 mass% or less, 0.10 mass% or more and 20 mass% or less, 0.10 mass% or more and 15 mass% or less, 0.10 mass% or more and 10 mass% or less, 0.30 mass% or more and 100 mass% or less, 0.30 mass% or more and 95 mass% or less, 0.30 mass% or more and 90 mass% or less, 0.30 mass% or more and 80 mass% or less, 0.30 mass% or more and 70 mass% or less, 0.30 mass% or more and 60 mass% or less, 0.30 mass% or more and 50 mass% or less, 0.30 mass% or more and 30 mass% or less, 0.30 mass% or more and 25 mass% or less, 0.30 mass% or more and 20 mass% or less, 0.30 mass% or more and 15 mass% or less, 0.30 mass% or more and 10 mass% or less, 1.0 mass% or more and 100 mass% or less, 1.0 mass% or more and 95 mass% or less, 1.0 mass% or more and 90 mass% or less, 1.0 mass% or more and 80 mass% or less, 1.0 mass% or more and 70 mass% or less, 1.0 mass% or more and 60 mass% or less, 1.0 mass% or more and 50 mass% or less, 1.0 mass% or more and 30 mass% or less, 1.0 mass% or more and 25 mass% or less, 1.0 mass% or more and 20 mass% or less, 1.0 mass% or more and 15 mass% or less, 1.0 mass% or more and 10 mass% or less, 1.5 mass% or more and 100 mass% or less, 1.5 mass% or more and 95 mass% or less,

1.5 mass% or more and 90 mass% or less, 1.5 mass% or more and 80 mass% or less, 1.5 mass% or more and 70 mass% or less, 1.5 mass% or more and 60 mass% or less, 1.5 mass% or more and 50 mass% or less, 1.5 mass% or more and 30 mass% or less, 1.5 mass% or more and 25 mass% or less, 1.5 mass% or more and 20 mass% or less, 1.5 mass% or more and 15 mass% or less, 1.5 mass% or more and 10 mass% or less, 2.0 mass% or more and 100 mass% or less, 2.0 mass% or more and 95 mass% or less, 2.0 mass% or more and 90 mass% or less, 2.0 mass% or more and 80 mass% or less, 2.0 mass% or more and 70 mass% or less, 2.0 mass% or more and 60 mass% or less, 2.0 mass% or more and 50 mass% or less, 2.0 mass% or more and 30 mass% or less, 2.0 mass% or more and 25 mass% or less, 2.0 mass% or more and 20 mass% or less, 2.0 mass% or more and 15 mass% or less, 2.0 mass% or more and 10 mass% or less, 2.5 mass% or more and 100 mass% or less, 2.5 mass% or more and 95 mass% or less, 2.5 mass% or more and 90 mass% or less, 2.5 mass% or more and 80 mass% or less, 2.5 mass% or more and 70 mass% or less, 2.5 mass% or more and 60 mass% or less, 2.5 mass% or more and 50 mass% or less, 2.5 mass% or more and 30 mass% or less, 2.5 mass% or more and 25 mass% or less, 2.5 mass% or more and 20 mass% or less, 2.5 mass% or more and 15 mass% or less, 2.5 mass% or more and 10 mass% or less, 3.0 mass% or more and 100 mass% or less, 3.0 mass% or more and 95 mass% or less, 3.0 mass% or more and 90 mass% or less, 3.0 mass% or more and 80 mass% or less, 3.0 mass% or more and 70 mass% or less, 3.0 mass% or more and 60 mass% or less, 3.0 mass% or more and 50 mass% or less, 3.0 mass% or more and 30 mass% or less, 3.0 mass% or more and 25 mass% or less, 3.0 mass% or

more and 20 mass% or less, 3.0 mass% or more and 15 mass% or less, 3.0 mass% or more and 10 mass% or less, 5.0 mass% or more and 100 mass% or less, 5.0 mass% or more and 95 mass% or less, 5.0 mass% or more and 90 mass% or less, 5.0 mass% or more and 80 mass% or less, 5.0 mass% or more and 70 mass% or less, 5.0 mass% or more and 60 mass% or less, 5.0 mass% or more and 50 mass% or less, 5.0 mass% or more and 30 mass% or less, 5.0 mass% or more and 25 mass% or less, 5.0 mass% or more and 20 mass% or less, 5.0 mass% or more and 15 mass% or less, 5.0 mass% or more and 10 mass% or less, 7.0 mass% or more and 100 mass% or less, 7.0 mass% or more and 95 mass% or less, 7.0 mass% or more and 90 mass% or less, 7.0 mass% or more and 80 mass% or less, 7.0 mass% or more and 70 mass% or less, 7.0 mass% or more and 60 mass% or less, 7.0 mass% or more and 50 mass% or less, 7.0 mass% or more and 30 mass% or less, 7.0 mass% or more and 25 mass% or less, 7.0 mass% or more and 20 mass% or less, 7.0 mass% or more and 15 mass% or less, 7.0 mass% or more and 10 mass% or less, 10.0 mass% or more and 100 mass% or less, 10.0 mass% or more and 95 mass% or less, 10.0 mass% or more and 90 mass% or less, 10.0 mass% or more and 80 mass% or less, 10.0 mass% or more and 70 mass% or less, 10.0 mass% or more and 60 mass% or less, 10.0 mass% or more and 50 mass% or less, 10.0 mass% or more and 30 mass% or less, 10.0 mass% or more and 25 mass% or less, 10.0 mass% or more and 20 mass% or less, 10.0 mass% or more and 15 mass% or less, 80.0 mass% or more and 100 mass% or less, 80.0 mass% or more and 95 mass% or less, 80.0 mass% or more and 90 mass% or less, 90.0 mass% or more and 100 mass% or less, 90.0 mass% or more and 95 mass% or

less, or 95.0 mass% or more and 100 mass% or less.

[0112] When the immunostimulatory composition of the present embodiment is in liquid form, the number of bacteria comprised in the composition according to the present embodiment may be 1.0×10^3 cells/mL or more, 1.0×10^4 cells/mL or more, 1.0×10^5 cells/mL or more, 1.0×10^6 cells/mL or more, 1.0×10^7 cells/mL or more, or 4.0×10^7 cells/mL or more, and may be 1.0×10^{11} cells/mL or less, 1.0×10^{10} cells/mL or less, 3.0×10^9 cells/mL or less, or 1.0×10^9 cells/mL or less. These lower limit values and upper limit values can each be combined arbitrarily and are not particularly limited. For example, the number of bacteria comprised in the composition according to the present embodiment may be 1.0×10^3 cells/mL or more and 1.0×10^{11} cells/mL or less, 1.0×10^3 cells/mL or more and 1.0×10^{10} cells/mL or less, 1.0×10^3 cells/mL or more and 3.0×10^9 cells/mL or less, 1.0×10^3 cells/mL or more and 1.0×10^9 cells/mL or less, 1.0×10^4 cells/mL or more and 1.0×10^{11} cells/mL or less, 1.0×10^4 cells/mL or more and 1.0×10^{10} cells/mL or less, 1.0×10^4 cells/mL or more and 3.0×10^9 cells/mL or less, 1.0×10^4 cells/mL or more and 1.0×10^9 cells/mL or less, 1.0×10^5 cells/mL or more and 1.0×10^{11} cells/mL or less, 1.0×10^5 cells/mL or more and 1.0×10^{10} cells/mL or less, 1.0×10^5 cells/mL or more and 3.0×10^9 cells/mL or less, 1.0×10^5 cells/mL or more and 1.0×10^9 cells/mL or less, 1.0×10^6 cells/mL or more and 1.0×10^{11} cells/mL or less, 1.0×10^6 cells/mL or more and 1.0×10^{10} cells/mL or less, 1.0×10^6 cells/mL or more and 3.0×10^9 cells/mL or less, 1.0×10^6 cells/mL or more and 1.0×10^9 cells/mL or less, 1.0×10^7 cells/mL or more and 1.0×10^{11} cells/mL or less, 1.0×10^7 cells/mL or more and 1.0×10^{10} cells/mL or less, 1.0×10^7 cells/mL or

more and 3.0×10^9 cells/mL or less, 1.0×10^7 cells/mL or more and 1.0×10^9 cells/mL or less, 4.0×10^7 cells/mL or more and 1.0×10^{11} cells/mL or less, 4.0×10^7 cells/mL or more and 1.0×10^{10} cells/mL or less, 4.0×10^7 cells/mL or more and 3.0×10^9 cells/mL or less, or 4.0×10^7 cells/mL or more and 1.0×10^9 cells/mL. In this case, the daily intake or dose of the liquid immunostimulatory composition may be 10 mL to 1,000 mL, 10 mL to 800 mL, 10 mL to 500 mL, 10 mL to 250 mL, 30 mL to 1,000 mL, 30 mL to 800 mL, 30 mL to 500 mL, 30 mL to 250 mL, 50 mL to 1,000 mL, 50 mL to 800 mL, 50 to 500 mL, 50 mL to 250 mL, 100 mL to 1,000 mL, 100 mL to 800 mL, 100 to 500 mL, or 100 to 250 mL.

[0113] In the immunostimulatory composition of the present embodiment, the number of bacteria according to the present embodiment comprised in the composition per unit package may be, for example, 1.0×10^4 or more, 5.0×10^4 or more, 1.0×10^5 or more, 3.0×10^5 or more, 1.0×10^6 or more, 3.0×10^6 or more, 1.0×10^7 or more, 3.0×10^7 or more, 1.0×10^8 or more, 3.0×10^8 or more, 5.0×10^8 or more, 1.0×10^9 or more, 5.0×10^9 or more, 1.0×10^{10} or more, 5.0×10^{10} or more, or 1.0×10^{11} or more, and may be 1.0×10^{14} or less, 1.0×10^{13} or less, or 1.0×10^{12} or less. These upper limit values and lower limit values can each be combined arbitrarily, and for example, the number of bacteria according to the present embodiment comprised in the composition per unit package in the immunostimulatory composition of the present embodiment may be, for example, 1.0×10^5 or more and 1.0×10^{14} or less, 1.0×10^5 or more and 1.0×10^{13} or less, 1.0×10^5 or more and 1.0×10^{12} or less, 3.0×10^5 or more and 1.0×10^{14} or less, 3.0×10^5 or more and 1.0×10^{13}

or less, 3.0×10^{5} or more and 1.0×10^{12} or less, 1.0×10^{6} or more and 1.0×10^{14} or less, 1.0×10^{6} or more and 1.0×10^{13} or less, 1.0×10^{6} or more and 1.0×10^{12} or less, 3.0×10^{6} or more and 1.0×10^{14} or less, 3.0×10^{6} or more and 1.0×10^{13} or less, 3.0×10^{6} or more and 1.0×10^{12} or less, 1.0×10^{7} or more and 1.0×10^{14} or less, 1.0×10^{7} or more and 1.0×10^{13} or less, 1.0×10^{7} or more and 1.0×10^{12} or less, 3.0×10^{7} or more and 1.0×10^{14} or less, 3.0×10^{7} or more and 1.0×10^{13} or less, 3.0×10^{7} or more and 1.0×10^{12} or less, 1.0×10^{8} or more and 1.0×10^{14} or less, 1.0×10^{8} or more and 1.0×10^{13} or less, 1.0×10^{8} or more and 1.0×10^{12} or less, 3.0×10^{8} or more and 1.0×10^{14} or less, 3.0×10^{8} or more and 1.0×10^{13} or less, 3.0×10^{8} or more and 1.0×10^{12} or less, 5.0×10^{8} or more and 1.0×10^{14} or less, 5.0×10^{8} or more and 1.0×10^{13} or less, 5.0×10^{8} or more and 1.0×10^{12} or less, 1.0×10^{9} or more and 1.0×10^{14} or less, 1.0×10^{9} or more and 1.0×10^{13} or less, 1.0×10^{9} or more and 1.0×10^{12} or less, 5.0×10^{9} or more and 1.0×10^{14} or less, 5.0×10^{9} or more and 1.0×10^{13} or less, 5.0×10^{9} or more and 1.0×10^{12} or less, 1.0×10^{10} or more and 1.0×10^{14} or less, 1.0×10^{10} or more and 1.0×10^{13} or less, 1.0×10^{10} or more and 1.0×10^{12} or less, 5.0×10^{10} or more and 1.0×10^{14} or less, 5.0×10^{10} or more and 1.0×10^{13} or less, 5.0×10^{10} or more and 1.0×10^{12} or less, 1.0×10^{11} or more and 1.0×10^{14} or less, 1.0×10^{11} or more and 1.0×10^{13} or less, or 1.0×10^{11} or more and 1.0×10^{12} or less.

[0114] The immunostimulatory composition of the present embodiment is preferably used by oral ingestion, oral administration, nasal ingestion, or nasal administration from the viewpoint of reducing the burden of ingestion or administration, and more preferably by oral ingestion or oral administration. When used by oral ingestion, oral administration, nasal

ingestion, or nasal administration, the bacterium or the composition comprising the bacterium preferably has high resistance to gastric juice, intestinal juice, and the like, and for example, preferably has strong acid resistance. The bacterium is not particularly limited, and either live bacteria or dead bacteria can be used, but from the viewpoints of immunostimulatory effect, stability, production efficiency, and the like, a dead bacterium is preferred, and a heat-killed bacterium is more preferred.

[0115] The immunostimulatory composition of the present embodiment can be orally fed to humans and non-human mammals, and a representative form of ingestion is a food composition. The food composition provided is a food composition comprising the bacterium according to the present embodiment in an effective amount. Here, "comprised in an effective amount" means a content such that when the amount normally consumed in each food composition is fed, the active ingredient of the present invention is fed to an extent that effects such as immunostimulation are exerted. In addition, the term "food composition" is used to include health foods, functional foods, nutritional supplements, foods with health claims (e.g., foods for specified health uses, foods with nutrient function claims, foods with function claims), foods for special dietary uses (e.g., foods for infants, foods for pregnant and nursing women, foods for the sick), and supplements. Note that when the active ingredient of the present invention is fed to mammals other than humans, it goes without saying that the food referred to in the present invention is used as feed.

[0116] Since the immunostimulatory composition of the present

embodiment has effects such as immunostimulation, it can be provided by being comprised in foods consumed on a daily basis. In this case, the immunostimulatory composition of the present embodiment can be provided in a unit package form in which the amount to be ingested per serving is predetermined. Examples of unit package forms per serving include forms that define a fixed amount in a pack, package, can, bottle, or the like. In order to better exert the various actions of the immunostimulatory composition of the present embodiment, the amount to be ingested per serving can be determined in accordance with the daily intake of the active ingredient of the present invention described below. The food of the present embodiment may be provided with instructions regarding the amount of ingestion displayed on the packaging, or together with a document or the like describing the instructions.

[0117] The predetermined amount to be ingested per serving in the unit package form may be the effective daily intake, or may be the effective daily intake divided into two or more (preferably two to six) portions. Therefore, the unit package form of the immunostimulatory composition of the present embodiment can comprise the active ingredient of the present invention in the daily intake described below, or can comprise the active ingredient of the present invention in an amount of 1/2 to 1/6 of the daily intake described below. The immunostimulatory composition of the present embodiment is preferably provided in a unit package form per serving (i.e., a unit package form per day) in which the amount to be ingested per serving is the effective daily intake, for the convenience of ingestion.

[0118] The immunostimulatory composition of the present embodiment

can be administered to a subject in need of immunostimulation. The subject in need of immunostimulation is not particularly limited, but examples include a subject infected with a virus, a subject with a cold, and a subject aged 65 or older.

[0119] The form of the "food composition" in the immunostimulatory composition of the present embodiment is not particularly limited, and may be, for example, in the form of a beverage, a semi-liquid or gel form, or a solid or powder form. In addition, as a "supplement," examples include tablets produced by adding excipients, binders, and the like to the active ingredient of the immunostimulatory composition of the present embodiment and kneading and then tableting, granules produced by adding excipients, binders, and the like to the active ingredient of the present invention and granulating, orally disintegrating tablets, and capsules produced by enclosing the active ingredient of the present invention in a capsule or the like. When provided as a supplement, in addition to the unit package form per serving or per day described above, it is also suitable to provide it in a unit package form per week, per two weeks, per month, or per two months. Note that in the latter unit package form, it is preferable that, for example, the amount to be ingested per serving or per day is displayed, and the person ingesting can ingest an effective amount of the active ingredient of the present invention according to the display.

[0120] Examples of food compositions provided as the immunostimulatory composition of the present embodiment include health foods, functional foods, nutritional compositions, nutritional supplements, supplements, foods for health use, foods for specified

health uses, foods with nutrient function claims, or foods with function claims, having immunostimulatory ability. Such food compositions can be labeled with, for example, support for maintaining immune function in healthy people (immune care), for those concerned about decreased immune function, suppressing decreased immune function, for those concerned about sunburn, for those concerned about skin damage in daily life, for those concerned about dry skin, for those concerned about skin flushing, for those concerned about skin erythema, for those concerned about skin redness, for those concerned about facial redness, for those concerned about rough hands, and the like.

[0121] The food composition provided as the immunostimulatory composition of the present embodiment is not particularly limited as long as it comprises the active ingredient of the present invention, but examples include soft drinks, carbonated beverages, fruit juice-containing beverages, vegetable juice-containing beverages, fruit juice and vegetable juice-containing beverages, daily milk such as cow's milk, soy milk, milk beverages, drink-type yogurt, drink-type or stick-type jelly, coffee, cocoa, tea beverages, nutritional drinks, energy drinks, sports drinks, mineral water (including both sparkling and non-sparkling), near water, non-alcoholic beer-taste beverages, and other non-alcoholic beverages; carbohydrate-containing foods and beverages such as cooked rice, noodles, bread, and pasta; dairy products such as cheese, hard-type or soft-type yogurt, fresh cream from daily milk or other fat raw materials, and ice cream; Western confectioneries such as cookies, cakes, and chocolate, Japanese confectioneries such as manju and yokan, tablet confectioneries (refreshing confectioneries) such as

ramune, candies, gums, gummies, cold confectioneries and frozen confectioneries such as jelly and pudding, and various confectioneries such as snack foods; alcoholic beverages such as whiskey, bourbon, spirits, liqueurs, wine, fruit wine, Japanese sake, Chinese liquor, shochu, beer, non-alcoholic beer with an alcohol content of 1% or less, happoshu, other miscellaneous liquors, and chuhai; processed foods such as processed egg products, processed marine products or livestock meat (including offal such as liver) (including delicacies), soups such as miso soup, seasonings such as miso, soy sauce, furikake, and other seasoning condiments, and liquid foods such as concentrated liquid foods.

[0122] Tea beverages include fermented teas, semi-fermented teas, and unfermented teas, and examples include black tea, green tea, barley tea, brown rice tea, sencha, gyokuro, hojicha, oolong tea, turmeric tea, pu-erh tea, rooibos tea, rose tea, chrysanthemum tea, ginkgo leaf tea, and herbal teas (e.g., mint tea, jasmine tea).

[0123] Examples of fruits used in fruit juice-containing beverages and fruit juice and vegetable juice-containing beverages include apples, mandarin oranges, grapes, bananas, pears, peaches, mangoes, acai, blueberries, and plums. In addition, examples of vegetables used in vegetable juice-containing beverages and fruit juice and vegetable juice-containing beverages include tomatoes, carrots, celery, pumpkins, cucumbers, and watermelons.

[0124] When the immunostimulatory composition of the present embodiment is provided as a feed, it can be implemented in accordance with the description regarding the above food.

[0125] When the immunostimulatory composition of the present

embodiment is provided as a pharmaceutical composition or a quasi-drug, it can be formulated and provided as an oral preparation or a parenteral preparation. Examples of oral preparations include granules, powders, tablets (including sugar-coated tablets), pills, capsules, syrups, liquids, jellies, emulsions, and suspensions. Examples of parenteral preparations include injections suitable for local administration (including intradermal injection, subcutaneous injection, intramuscular injection, and intravenous injection), inhalants (e.g., inhalation aerosols, inhalation powders, inhalation liquids), nasal drops (e.g., nasal powders, nasal liquids), ointments, creams, gels, suppositories, patches, and poultices, and in one aspect may be nasal drops. These preparations can be formulated using pharmaceutically acceptable carriers by methods commonly performed in the art. Examples of pharmaceutically acceptable carriers include excipients, binders, diluents, additives, fragrances, buffers, thickeners, colorants, stabilizers, emulsifiers, dispersants, suspending agents, and preservatives.

[0126] When the immunostimulatory composition of the present embodiment is used as a pharmaceutical composition or a quasi-drug, examples of target diseases include diseases already known as indications for Type I IFN, such as cancers including renal cancer, multiple myeloma, chronic myelogenous leukemia, hairy cell leukemia, glioblastoma, medulloblastoma, astrocytoma, malignant melanoma, mycosis fungoides, and adult T-cell leukemia; viral infections including subacute sclerosing panencephalitis, HTLV-1 myelopathy, hepatitis B, and hepatitis C; bacterial infections caused by Chlamydia (sexually transmitted disease), Mycobacterium (tuberculosis), Listeria (sepsis,

etc.), Staphylococcus (food poisoning), and Helicobacter (gastritis); and autoimmune diseases including multiple sclerosis. The immunostimulatory composition of the present embodiment can be used for the prevention or treatment of the above diseases. In addition, since Type I IFN is known to have the function of inhibiting differentiation from osteoblasts to osteoclasts, the immunostimulatory composition of the present embodiment can also be used for the prevention or treatment of osteoporosis and the like.

[0127] The immunostimulatory composition of the present embodiment can be used as a vaccine by expressing an antigen corresponding to a specific disease in or on the surface of the bacterium as an active ingredient, or by secreting it outside the bacterium, using genetic engineering techniques. In particular, since the cell wall of bacteria has the function of protecting antigens from gastric acid, a heterologous antigen-expressing bacterial strain in which an antigen is expressed in or on the surface of bacteria is suitable as a host for oral vaccines.

[0128] The immunostimulatory composition of the present embodiment may be a bacterial powder of the bacterium according to the present embodiment (a powder obtained by drying bacterial cells or a powder containing the same, etc.). Such a bacterial powder can be obtained, for example, by appropriately culturing, sterilizing, and drying bacteria according to conventional production methods. In addition, the immunostimulatory composition of the present embodiment may be, for example, an immunostimulatory composition produced according to the production method according to one aspect of the second embodiment.

[0129] The bacterial powder of the present embodiment may contain, in

addition to the bacterium according to the present embodiment and components derived from the culture medium during bacterial culture, components acceptable as food, pharmaceutical, or feed, and may further contain, for example, at least one selected from the group consisting of sugars, proteins, lipids, amino acids, vitamins, minerals, flavonoids, quinones, polyphenols, nucleic acids, fatty acids, acidulants, sweeteners, colorants, fragrances, seasonings, table salt, emulsifiers, stabilizers, cooling agents, binders, disintegrants, lubricants, colorants, preservatives, sustained-release modifiers, surfactants, and solubilizers.

[0130] The bacterial powder of the present embodiment can be used as a raw material for the food composition, pharmaceutical composition, or feed of the present embodiment.

[0131] When the immunostimulatory composition of the present embodiment is provided as an additive, it can be implemented in accordance with the description regarding the above food composition, feed, quasi-drug, pharmaceutical composition, or bacterial powder. When the immunostimulatory composition of the present embodiment is provided as a food additive, the immunostimulatory composition of the present embodiment can be used as a functional ingredient in foods with function claims having an immunostimulatory effect.

[0132] The immunostimulatory composition of the present embodiment can be determined depending on the sex, age, and body weight of the recipient, symptoms, ingestion time or administration time, dosage form, ingestion route or administration route, and materials or drugs to be combined. The daily intake or dose per adult of the immunostimulatory composition of the present embodiment can be specified, for example,

by the number of bacteria as the active ingredient, and can be 1×10^{8} or more, 1×10^{9} or more, or 1×10^{10} or more, and can be 1×10^{14} or less, 1×10^{13} or less, or 1×10^{12} or less. These upper limit values and lower limit values can each be combined arbitrarily, and the range of the above intake or dose can be, for example, 1×10^{8} to 1×10^{14}, 1×10^{9} to 1×10^{14}, or 1×10^{10} to 1×10^{14}, 1×10^{8} to 1×10^{13}, 1×10^{9} to 1×10^{13}, 1×10^{10} to 1×10^{13}, 1×10^{8} to 1×10^{12}, 1×10^{9} to 1×10^{12}, or 1×10^{10} to 1×10^{12}. The number of bacteria can be measured by a known microscope, flow cytometer, or non-culture rapid microbial testing device (e.g., ELESTA PixeeMo (AFI Technology Co., Ltd.)) and the like, but measurement by microscope is preferred from the viewpoint of versatility. The subject to which the composition according to the present embodiment is administered or that ingests the composition may be a human and/or a non-human mammal, and may be a human.

[0133] The daily intake or dose per adult of the immunostimulatory composition of the present embodiment can also be specified by the dry cell mass of the bacterium as the active ingredient, and can be, for example, 2.5×10^{-2} mg or more, 2.5×10^{-1} mg or more, or 2.5 mg or more, and can be 2.5×10^{4} mg or less, 2.5×10^{3} mg or less, or 2.5×10^{2} mg or less. These upper limit values and lower limit values can each be combined arbitrarily, and the range of the above intake or dose can be, for example, 2.5×10^{-2} mg to 2.5×10^{4} mg, 2.5×10^{-1} mg to 2.5×10^{4} mg, 2.5 mg to 2.5×10^{4} mg, 2.5×10^{-2} mg to 2.5×10^{3} mg, 2.5×10^{-1} mg to 2.5×10^{3} mg, 2.5×10 mg to 2.5×10^{3} mg, 2.5×10^{-2} mg to 2.5×10^{2} mg, 2.5×10^{-1} mg to 2.5×10^{2} mg, or 2.5 mg to 2.5×10^{2} mg.

[0134] The intake amount and the following ingestion timing and

ingestion period of the immunostimulatory composition of the present embodiment apply to both non-therapeutic and therapeutic use of the immunostimulatory composition of the present embodiment, and in the case of therapeutic use, ingestion can be read as administration.

[0135] The immunostimulatory composition of the present embodiment is preferably fed or administered continuously during the period in which an immunostimulatory effect is expected. The ingestion period or administration period of the active ingredient of the present invention can be, for example, 1 week or more, 2 weeks or more, 3 weeks or more, preferably 1 month or more (4 weeks or more) of ingestion or administration at the above daily dose, from the viewpoint of better exerting the immunostimulatory effect. The ingestion interval or administration interval of the active ingredient of the present invention can be once every 3 days, once every 2 days, or once a day at the above daily dose, and is preferably once a day.

[0136] The immunostimulatory composition of the present embodiment may also be started to be fed or administered before an event or period in which an immunostimulatory effect is expected. Examples of events in which an immunostimulatory effect is expected include actions that may result in infection with a virus (e.g., participation in events with a high risk of viral infection, travel to endemic areas), and examples of periods in which an immunostimulatory effect is expected include epidemic periods of viral infection. Examples of ingestion timing or administration timing before an event in which an immunostimulatory effect is expected include 1 day or more before, 3 days or more before, 1 week or more before, 2 weeks or more before, 3 weeks or more before,

1 month or more before (4 weeks or more before), or 2 months or more before (8 weeks or more before). In addition, although not particularly limited, from the start of ingestion or administration until the event in which an immunostimulatory effect is expected, ingestion or administration may be continued with ingestion intervals or administration intervals in some cases. The active ingredient of the present invention may also be started to be ingested or administered after an event or period in which an immunostimulatory effect is expected. Examples of ingestion timing or administration timing after an event in which an immunostimulatory effect is expected include 1 day or more after, 3 days or more after, 1 week or more after, and 2 weeks or more after. In addition, although not particularly limited, when feeding or administering after an event in which an immunostimulatory effect is expected, ingestion or administration may be continued with ingestion intervals or administration intervals in some cases. In the present invention, it is particularly preferred to start ingestion or administration of the active ingredient of the present invention before an event in which an immunostimulatory effect is expected, and to continue ingestion or administration until after the event.

[0137] Note that the aspect in which the composition is an immunostimulatory composition in the present embodiment can also be said, from another perspective, to be a method for stimulating immunity of a subject, comprising feeding to a subject or administering to a subject the composition according to one aspect of the present embodiment. The subject fed to or administered to by the composition may be a subject in need thereof, and may be, for example, a subject in need of

immunostimulation as described above.

[0138] In addition, the aspect in which the composition is an immunostimulatory composition in the present embodiment can also be said, from another perspective, to be use of the composition according to one aspect of the present embodiment in stimulating immunity of a subject. The use in this case may be non-therapeutic use and/or therapeutic use, and is preferably non-therapeutic use.

[0139] In the present disclosure, “therapeutic” use refers to use in treating the body of a human or animal. The therapeutic use according to the present disclosure may be, for example, use for the purpose of medical practice or involving medical practice. The therapeutic use according to the present disclosure may involve, for example, a medical professional administering or feeding a substance a human or animal, or instructing a human or animal to administer or ingest a substance. The therapeutic use according to the present disclosure may be, for example, use for therapeutic purposes or preventive purposes, and may be use for therapeutic purposes or preventive purposes involving ingestion of a food composition, and may be for therapeutic purposes. The therapeutic use according to the present disclosure may be, for example, use for non-healthy individuals.

[0140] In the present disclosure, “non-therapeutic” use means use of a substance that does not fall under therapeutic use. The non-therapeutic use according to the present disclosure may be, for example, use that is not for the purpose of medical practice and/or does not involve medical practice. The non-therapeutic use according to the present disclosure need not involve, for example, a medical professional administering or

feeding a substance to a human or animal, and/or instructing a human or animal to administer or ingest a substance. The non-therapeutic use according to the present disclosure may be, for example, use for preventive purposes or health promotion purposes, and may be use for preventive purposes involving administration or ingestion of a pharmaceutical composition or a quasi-drug, or use for health promotion purposes. The non-therapeutic use according to the present disclosure may be, for example, use for healthy individuals.

[0141] In addition, the aspect in which the composition is an immunostimulatory composition in the present embodiment can also be said, from another perspective, to be the composition according to one aspect of the present embodiment for use in stimulation of immunity of a subject. The use in this case may be, for example, therapeutic use.

[0142] In addition, the aspect in which the composition is an immunostimulatory composition in the present embodiment can also be said, from another perspective, to be use of the above bacterium in the manufacture of the composition according to one aspect of the present embodiment.

[0143] In addition, one aspect of the present embodiment is a method for activating a pDC, characterized by binding a bacterium to BDCA2.

[0144] In one aspect, for the biological characteristics, classification, and strains of bacteria that can be used in the above method, the description of the bacterium comprised in the immunostimulatory composition of the present embodiment (the “bacterium according to the present embodiment”) described above isapplied. For example, the above bacterium may be a lactic acid bacterium and/or an acetic acid

bacterium. In addition, the above bacterium may be a bacterium that is phagocytosed by a pDC via binding to BDCA2.

[0145] In addition, one aspect of the present embodiment is a method for activating a pDC, comprising administering to a subject a bacterium that binds to BDCA2.

[0146] In one aspect, for the description of the biological characteristics, classification, and strains of bacteria that can be used in the above method, the description of the bacterium comprised in the immunostimulatory composition of the present embodiment (the "bacterium according to the present embodiment") described above isapplied. For example, the above bacterium may be a lactic acid bacterium and/or an acetic acid bacterium. In addition, the above bacterium may be a bacterium that is phagocytosed by a pDC via binding to BDCA2.

[0147] In one aspect, the subject of the above method may be a human. The human is not particularly limited, but may be, for example, a subject in need of immunostimulation. The subject in need of immunostimulation is not particularly limited, but examples include a subject infected with a virus, a subject with a cold, and a subject aged 65 or older.

[0148] In addition, one aspect of the present embodiment may be a composition comprising a bacterium for use in the above "method for activating a pDC, characterized by binding a bacterium to BDCA2" and "method for activating a pDC, comprising administering to a subject a bacterium that binds to BDCA2."

[0149] In one aspect, for the description of the biological characteristics,

classification, and strains of bacteria that can be used in the above composition, the description of the bacterium comprised in the immunostimulatory composition of the present embodiment (the "bacterium according to the present embodiment") described above is applied. For example, the above bacterium may be a lactic acid bacterium and/or an acetic acid bacterium. In addition, for example, the above bacterium may be *Lactococcus lactis* subsp. *lactis* JCM 5805. In addition, the above bacterium may be a bacterium that is phagocytosed by a pDC via binding to BDCA2. In addition, for the description of the above composition, the description of the "immunostimulatory composition of the present embodiment" described as one embodiment of the present invention is applied.

[0150] The second embodiment of the present disclosure relates to a method for enhancing immunostimulatory ability and/or susceptibility to phagocytosis by a plasmacytoid dendritic cell (pDC) of a bacterium, comprising a step of culturing the bacterium in M17 medium supplemented with glucose at a predetermined concentration (culture step). In the method of the second embodiment, by using M17 medium supplemented with glucose at a predetermined concentration as the medium for culturing bacteria, the immunostimulatory ability and/or susceptibility to phagocytosis by a pDC of the bacteria tends to be higher compared to when such medium is not used.

[0151] M17 medium can be obtained from suppliers such as Becton, Dickinson and Company and the like. M17 medium is, for example, a medium in which the solvent is water and which contains, as solutes, yeast extract, peptone, tryptone, meat extract, lactose, ascorbic acid,

magnesium sulfate, and sodium glycerophosphate. M17 medium may be adjusted to a pH of 7.1±0.2 at the time of use, for example.

[0152] The final concentration of glucose in the medium used in the culture step may be, for example, 0.1 mass% or more, 0.3 mass% or more, 0.5 mass% or more, or 1.0 mass% or more, and may be 10 mass% or less, 5.0 mass% or less, 3.0 mass% or less, or 1.0 mass% or less. These lower limit values and upper limit values can be combined arbitrarily, and the final concentration of glucose in the medium used in the culture step may be, for example, 0.1 mass% or more and 10 mass% or less, 0.3 mass% or more and 10 mass% or less, 0.5 mass% or more and 10 mass% or less, 1.0 mass% or more and 10 mass% or less, 0.1 mass% or more and 5.0 mass% or less, 0.3 mass% or more and 5.0 mass% or less, 0.5 mass% or more and 5.0 mass% or less, 1.0 mass% or more and 5.0 mass% or less, 0.1 mass% or more and 3.0 mass% or less, 0.3 mass% or more and 3.0 mass% or less, 0.5 mass% or more and 3.0 mass% or less, 1.0 mass% or more and 3.0 mass% or less, 0.1 mass% or more and 1.0 mass% or less, 0.3 mass% or more and 1.0 mass% or less, 0.5 mass% or more and 1.0 mass% or less, or 1.0 mass%. Note that the glucose in this case is, for example, D-glucose.

[0153] The culture time in the culture step is not particularly limited, but may be, for example, 1 hour or more, 3 hours or more, 6 hours or more, 12 hours or more, or 18 hours or more, and may be 336 hours or less, 168 hours or less, 72 hours or less, 48 hours or less, or 36 hours or less, and may be, for example, 24 hours. The culture temperature in the culture step is not particularly limited, but may be, for example, 20°C or more, 22°C or more, 25°C or more, or 30°C or more, and may be 45°C

or less, 40℃ or less, 38℃ or less, 35℃ or less, or 30℃ or less, and may be, for example, 30℃.

[0154] The immunostimulatory ability and/or susceptibility to phagocytosis by a pDC of bacteria may be evaluated in the same manner as described in the first embodiment. The bacterium after the culture step may have an immunostimulatory ability and/or susceptibility to phagocytosis by a pDC that is, for example, 1.05 times or more, 1.10 times or more, 1.15 times or more, 1.20 times or more, 1.25 times or more, 1.30 times or more, 1.35 times or more, 1.40 times or more, 1.45 times or more, or 1.50 times or more that of the bacterium when the culture step is performed using MRS medium as the medium.

[0155] One aspect of the second embodiment may also be a method for producing an immunostimulatory composition, comprising the culture step. The immunostimulatory composition produced by the production method of the present aspect may be the composition of the first embodiment, and for example, its use and composition may satisfy the requirements for the composition of the first embodiment. The production method of the present aspect may further comprise, after the culture step, a step of sterilizing the bacteria and/or a step of drying the bacteria.

[0156] The sterilization method in the step of sterilizing bacteria may be a method commonly used by those skilled in the art, and may be, for example, heat treatment, pressure treatment, high-pressure steam treatment, electromagnetic wave treatment, electron beam treatment, radiation treatment, ultraviolet treatment, alcohol treatment, or electrolyzed water treatment, and in one aspect may be heat treatment.

When the sterilization method is heat treatment, the heating temperature is not particularly limited, but may be, for example, 60℃ or more, 65℃ or more, 70℃ or more, or 75℃ or more, and may be 110℃ or less, 100℃ or less, 90℃ or less, 85℃ or less, or 80℃ or less. These lower limit values and upper limit values can each be combined arbitrarily, and for example, the heating temperature may be 60℃ or more and 110℃ or less, 60℃ or more and 100℃ or less, 60℃ or more and 90℃ or less, 60℃ or more and 85℃ or less, 60℃ or more and 80℃ or less, 65℃ or more and 100℃ or less, 65℃ or more and 90℃ or less, 65℃ or more and 85℃ or less, 65℃ or more and 80℃ or less, 70℃ or more and 90℃ or less, 70℃ or more and 85℃ or less, 70℃ or more and 80℃ or less, 75℃ or more and 90℃ or less, or 75℃ or more and 85℃ or less. In addition, when the sterilization method is heat treatment, the heating time is not particularly limited, but may be, for example, 1 minute or more, 3 minutes or more, 5 minutes or more, or 10 minutes or more, and may be 24 hours or less, 4 hours or less, 60 minutes or less, 45 minutes or less, or 30 minutes or less. These lower limit values and upper limit values can each be combined arbitrarily, and for example, the heat treatment time may be 1 minute or more and 24 hours or less, 3 minutes or more and 4 hours or less, or 5 minutes or more and 60 minutes or less.

[0157] The drying method in the step of drying bacteria may be a method commonly used by those skilled in the art, and may be, for example, freeze-drying, spray drying, drum drying, hot air drying, or vacuum drying, and in one aspect may be spray drying.

[0158] In addition, the production method of the present aspect may comprise, between and/or during each step, a step of washing bacteria as

necessary, and such washing may be, for example, suspending bacteria in fresh medium or buffer and then isolating by centrifugation or the like.

[0159] The immunostimulatory composition produced by the production method of the present aspect may be, for example, an immunostimulatory composition comprising *Lactococcus lactis* subsp. *lactis* JCM 5805 as an active ingredient. The *Lactococcus lactis* subsp. *lactis* JCM 5805 in this case may satisfy, for example, the following Requirement Y, and satisfaction of Requirement Y may be confirmed in accordance with the method described in relation to the first embodiment.

[Requirement Y] After pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

Examples

[0160] The present invention will be described in more detail below using Examples, but the present invention is not limited to the following Examples.

[0161] In the following Examples, CAL-1 cells (accession number FERM BP-10914) provided by Nagasaki University were used as samples (hereinafter referred to as the present samples) (all rights to the present samples belong to Nagasaki University, and a license to use the present samples was obtained from Nagasaki University). Cell culture

was performed at 37°C and 5% CO_2. Results are shown as mean ± standard deviation. As the basal medium for culturing CAL-1 cells and for tests using CAL-1 cells, RPMI-1640 medium (Sigma, R8758) supplemented with penicillin/streptomycin (Gibco, 15140-12) at a final concentration of 1.0% by volume was used (hereinafter also referred to as "serum-free medium").

[0162] The strains of lactic acid bacteria used in the following Examples are shown in Table 1. The culture conditions shown as A to G in Table 1 are shown in Table 2. In the Examples, lactic acid bacteria, except for *Lactobacillus rhamnosus* CRL1505, were cultured according to the culture methods described in Table 2 in accordance with the correspondence of A to G shown in Table 1, unless otherwise specified, and then used as bacterial powder (a powder obtained by drying bacterial cells or a powder containing the same, etc.) prepared as follows. Bacteria cultured in MRS medium (MRS BROTH, CODE: CM0359, Oxoid) were centrifuged (5000 rpm), the supernatant was removed, and a bacterial suspension was obtained. Subsequently, the obtained bacterial suspension and phosphate-buffered saline (Bio) were mixed at a ratio of 1:19, and washed twice by centrifugation (5000 rpm). The washed bacterial suspension was heat-treated to kill the lactic acid bacteria in the bacterial suspension. The heat treatment was performed by raising the temperature from room temperature to 80°C over 30 minutes, maintaining at 80°C for 30 minutes, and then lowering the temperature to room temperature over 30 minutes. After cooling, freeze-drying was performed to obtain bacterial powder.

[0163] [Table 1]

Name of Bacterium	Notation	Culture

	in Examples	condition
Lactococcus plantarum JCM 11056	JCM 11056	B
Lactococcus curvatus JCM 1096	JCM 1096	B
Lactococcus kefiranofaciens JCM 6985	JCM 6985	H
Lactococcus murinus JCM 1717	JCM 1717	A
Lactococcus parakefiri JCM 8573	JCM 8573	I
Lactococcus paraplantarum JCM 12533	JCM 12533	C
Leuconostoc carnosum JCM 9695	JCM 9695	B
Leuconostoc citreum JCM 9698	JCM 9698	B
Streptococcus salivarius JCM 5707	JCM 5707	A
Enterococcus lactis JCM 30200	JCM 30200	A
Tetragenococcus halophilus ATCC 33315	ATCC 33315	F
Oenococcus oeni JCM 6125	JCM 6125	G
Weissella paramesenteroides JCM 9890	JCM 9890	B
Weissella viridescens JCM 1174	JCM 1174	A
Bifidobacterium animalis subsp. *animalis* JCM 1190	JCM 1190	E
Bifidobacterium animalis subsp. *lactis* JCM 10602	JCM 10602	E
Bifidobacterium bifidum JCM 1255	JCM 1255	J
Bifidobacterium longum subsp. *longum* JCM 1217	JCM 1217	E
Bifidobacterium longum subsp. *infantis* JCM 1222	JCM 1222	E
Bifidobacterium mongoliense JCM 15461	JCM 15461	D
Bifidobacterium pseudolongum JCM 1205	JCM 1205	E
Bifidobacterium reuteri JCM 17295	JCM 17295	E
Lactococcus lactis subsp. *lactis* JCM 5805	LC-Plasma	B
Lactococcus lactis subsp. *lactis* ATCC IL1403	ATCC IL1403	B
Lactococcus lactis subsp. *lactis* ATCC 7963	ATCC 7963	B
Lactococcus lactis subsp. *lactis* ATCC 7962	ATCC 7962	B
Lactococcus lactis subsp. *lactis* ATCC 29146	ATCC 29146	B
Lactococcus lactis subsp. *lactis* ATCC 27861	ATCC 27861	B
Lactococcus lactis subsp. *lactis* ATCC 19435	ATCC 19435	B
Lactococcus lactis subsp. *lactis* ATCC 15577	ATCC 15577	B
Lactococcus lactis subsp. *lactis* ATCC 15346	ATCC 15346	B
Lactococcus lactis subsp. *lactis* ATCC 13675	ATCC 13675	B
Lactococcus lactis subsp. *lactis* ATCC 12929	ATCC 12929	B
Lactococcus lactis subsp. *lactis* ATCC 11955	ATCC 11955	B

Lactococcus lactis subsp. *lactis* ATCC 11454	ATCC 11454	B
Lactococcus lactis subsp. *lactis* ATCC 11007	ATCC 11007	B
Lactobacillus rhamnosus CRL1505	CRL1505	(Not cultured)

[0164] [Table 2]

Condition	Culture method
Condition A	The bacterial cells of the corresponding strain number were suspended in MRS medium (product name: Difco Lactobacilli MRS Broth, manufacturer: Becton, Dickinson and Company; the same applies hereinafter) and seeded on MRS agar medium. After culturing at 37°C for 48 hours, colony picking was performed, and the cells were seeded in 10 mL of MRS medium and cultured at 37°C for 24 hours. 1 mL of the cultured bacterial suspension was seeded in 100 mL of MRS medium and cultured at 37°C for 24 hours.
Condition B	The bacterial cells of the corresponding strain number were suspended in MRS medium and seeded on MRS agar medium. After culturing at 30°C for 48 hours, colony picking was performed, and the cells were seeded in 10 mL of MRS medium and cultured at 30°C for 24 hours. 1 mL of the cultured bacterial suspension was seeded in 100 mL of MRS medium and cultured at 30°C for 24 hours.
Condition C	The bacterial cells of the corresponding strain number were suspended in MRS medium and seeded on MRS agar medium. After culturing at 30°C for 48 hours under anaerobic conditions, colony picking was performed, and the cells were seeded in 10 mL of MRS medium and cultured at 30°C for 24 hours under anaerobic conditions. 1 mL of the cultured bacterial suspension was seeded in 100 mL of MRS medium and cultured at 30°C for 24 hours under anaerobic conditions.
Condition D	The bacterial cells of the corresponding strain number were suspended in lactose-supplemented GAM medium (GAM medium (product name: GAM Bouillon "Nissui", manufacturer: Nissui Pharmaceutical Co., Ltd.; the same applies hereinafter) supplemented with 1 mass% lactose) and seeded on lactose-supplemented GAM agar medium (GAM agar medium supplemented with 1 mass% lactose). After culturing at 30°C for 48 hours under anaerobic conditions, colony picking was performed, and the cells were seeded in 10 mL of lactose-supplemented GAM medium and cultured at 30°C for 24 hours under anaerobic conditions. 1 mL of the cultured bacterial suspension was seeded in lactose-supplemented GAM medium and cultured at 30°C for 24 hours under anaerobic conditions.
Condition E	The bacterial cells of the corresponding strain number were suspended in GAM medium and seeded on GAM agar medium. After culturing at 37°C for 48 hours under anaerobic conditions, colony picking was performed, and the cells were seeded in 10 mL of GAM medium and cultured at 37°C for 24 hours under anaerobic conditions. 1 mL of the cultured bacterial suspension was seeded in 100 mL of GAM medium and cultured at 37°C for 24 hours under anaerobic conditions.

Condition F	The bacterial cells of the corresponding strain number were suspended in the following NaCl-supplemented Yeast Extract medium (Yeast Extract (product name: Bacto Yeast Extract, distributor: Thermo Fisher Scientific K.K.; the same applies hereinafter) medium supplemented with 5 mass% NaCl) and seeded on NaCl-supplemented yeast agar medium (yeast agar medium supplemented with 5 mass% NaCl). After culturing at 37°C for 48 hours, colony picking was performed, and the cells were seeded in 10 mL of NaCl-supplemented Yeast Extract medium and cultured at 37°C for 24 hours. The entire amount of the cultured bacterial suspension concentrated to 1 mL by centrifugation was seeded in 100 mL of NaCl-supplemented Yeast Extract medium and cultured at 37°C for 96 hours.
Condition G	The bacterial cells of the corresponding strain number were suspended in MRS medium and seeded on tomato medium agar medium (MRS medium supplemented with 10 mass% tomato juice supernatant (centrifuged twice at 3300 g for 10 min at 4°C to remove solids) and adjusted to pH 4.8). After culturing at 37°C for 48 hours, colony picking was performed, and the cells were seeded in 10 mL of tomato medium and cultured at 37°C for 24 hours. 1 mL of the cultured bacterial suspension was seeded in 100 mL of tomato medium and cultured at 37°C for 24 hours under anaerobic conditions.
Condition H	The bacterial cells of the corresponding strain number were suspended in MRS (pH 5.5) medium and seeded on MRS (pH 5.5) agar medium. After culturing at 30°C for 48 hours, colony picking was performed, and the cells were seeded in 10 mL of MRS (pH 5.5) medium and cultured at 30°C for 24 hours. 1 mL of the cultured bacterial suspension was seeded in 100 mL of MRS (pH 5.5) medium and cultured at 30°C for 96 hours.
Condition I	The bacterial cells of the corresponding strain number were suspended in MRS (pH 5.5) medium and seeded on MRS (pH 5.5) agar medium. After culturing at 30°C for 48 hours, colony picking was performed, and the cells were seeded in 10 mL of MRS (pH 5.5) medium and cultured at 30°C for 24 hours. 1 mL of the cultured bacterial suspension was seeded in 100 mL of MRS (pH 5.5) medium and cultured at 30°C for 24 hours.
Condition J	The bacterial cells of the corresponding strain number were suspended in GAM medium and seeded on GAM agar medium. After culturing at 37°C for 48 hours under anaerobic conditions, colony picking was performed, and the cells were seeded in 10 mL of GAM medium and cultured at 37°C for 24 hours under anaerobic conditions. The entire amount of the cultured bacterial suspension concentrated to 1 mL by centrifugation was seeded in 100 mL of GAM medium and cultured at 37°C for 96 hours under anaerobic conditions.

[0165] For *Lactobacillus rhamnosus* CRL1505, bacterial powder prepared as follows was used.

[Method for preparing bacterial powder of *Lactobacillus rhamnosus* CRL1505]

A supplement containing *Lactobacillus rhamnosus* CRL1505,

FLORASSIST Probiotic Immune & Nasal Defense (manufactured by Life Extension, #02208), was dissolved in 10 mL of MRS medium to prepare a solution containing *Lactobacillus rhamnosus* CRL1505. The solution was spread on MRS agar medium and streak culture was performed to obtain single-colony *Lactobacillus rhamnosus* CRL1505. A colony of *Lactobacillus rhamnosus* CRL1505 was added to MRS medium and incubated at 37°C for 24 hours, and 700 μL of the culture solution was mixed with 300 μL of 80% glycerol to prepare a glycerol stock of *Lactobacillus rhamnosus* CRL1505, which was stored at -80°C. 100 μL of the glycerol stock of *Lactobacillus rhamnosus* CRL1505 was added to 100 mL of MRS medium and incubated at 37°C for 24 hours. The culture solution was collected, washed twice by centrifugation and washing with ultrapure water, then suspended in 20 mL of ultrapure water, sterilized at 80°C for 30 minutes, and then freeze-dried, and this was used as the bacterial powder of *Lactobacillus rhamnosus* CRL1505 in the following Examples.

[0166] [Staining of bacteria with pHrodo Red SE]

pHrodo Red SE (Invitrogen, P36600) was dissolved in DMSO to prepare a 10.2 mM solution. The bacterial powder was weighed into a 2 mL Eppendorf tube, and 0.1 M sodium bicarbonate adjusted to pH 9.0 was added to prepare a solution of 20 mg/mL bacteria. Thereafter, 95 μL was transferred to a new 2 mL Eppendorf tube, and 5 μL of the 10.2 mM pHrodo Red SE prepared as described above was added. After dispersing by vortexing, the tube was shielded from light with aluminum foil and incubated at room temperature for 60 minutes. Thereafter, 750 μL of PBS (Takara Bio) was added, and the mixture was vortexed and centrifuged

at 20,000 g×2 minutes at room temperature. After discarding 800 μL of the supernatant, 1.5 mL of PBS was added, and the pellet was completely resuspended by vortexing. After the mixture was centrifuged again at 20,000 g×2 minutes at room temperature, 1.5 mL of the supernatant was discarded. Finally, 140 μL of PBS was added and the pellet was resuspended to prepare a bacterial suspension of 10 mg/mL stained bacteria. The stained bacterial suspension was stored shielded from light with aluminum foil at 4℃ until use. Immediately before use, the suspension was diluted 10-fold with PBS and used as a bacterial suspension of 1 mg/mL bacteria. Note that the stained bacterial suspension was used within 24 hours of preparation.

[0167] <Example 1: Evaluation of the contribution of phagocytosis to IFN-α production by pDCs>

A cell suspension containing CAL-1 cells at 5.0×10^5 cells/mL was prepared using serum-free medium, seeded at 10 mL/well in a 10 cm diameter culture dish, and cultured for 16 hours (pre-treatment step). CAL-1 cells were collected by pipetting. The collected CAL-1 cells were resuspended in serum-free medium at a concentration of 2.0×10^5 cells/mL and seeded at 200 μL/well in each well of a 96-well microplate. To the cell suspension in each of those wells, in the LC-Plasma+Cyt_D group, 400 μM cytochalasin D (Fujifilm Wako, 037-17561) dissolved in 4% by volume dimethyl sulfoxide (DMSO) was prepared, and cytochalasin D and DMSO were added at final concentrations of 10 μM and 0.1% by volume, respectively, followed by incubation for 30 minutes. In the LC-Plasma+DMSO group, 4% by volume DMSO was added at a final concentration of 0.1% by volume, followed by

incubation for 30 minutes in the same manner. To each well after incubation, *Lactococcus lactis* subsp. *lactis* JCM 5805 (Kirin Holdings Co., Ltd., hereinafter also referred to as "LC-Plasma") stained with pHrodo Red SE (Invitrogen, P36600) by the method of the above-described [Staining of bacteria with pHrodo Red SE] was added at a final concentration of 10 μg/mL. Thereafter, the fluorescence of pHrodo Red SE in each well was measured over time using an Incucyte(registered trademark) SX5 Live-Cell Analysis System (Sartorius). Furthermore, after culturing for 22 hours (contact step), the culture supernatant of each well was collected, and the IFN-α concentration in the culture supernatant was measured by ELISA using a Human IFN-Alpha Multi-Subtype ELISA Kit (PBL Assay Science).

[0168] Note that cytochalasin D is an actin polymerization inhibitor, and it is known that phagocytosis is suppressed in cells to which cytochalasin D has been added. In addition, pHrodo Red SE is a staining reagent whose fluorescence intensity is enhanced in a low pH environment. When bacteria stained with pHrodo Red SE are phagocytosed by CAL-1 cells, the bacteria enter endosomes and the fluorescence intensity of pHrodo Red SE increases due to the low pH environment. Therefore, the amount of phagocytosis of bacteria by CAL-1 cells can be quantified using the total area in which fluorescence of pHrodo Red SE is detected (fluorescence detection area) in the entire field of view as an indicator. Note that the fluorescence intensity analysis conditions of the Incucyte(registered trademark) SX5 Live-Cell Analysis System (Sartorius) were as described in the following [Incucyte fluorescence intensity analysis condition 1]. The field of view area per image was

0.572 mm^2.

[0169] [Incucyte fluorescence intensity analysis condition 1]

In the Incucyte fluorescence intensity analysis, two channels were set: "Phase channel" and "Orange channel." In the Phase channel, CAL-1 cells were detected based on phase contrast. In the Orange channel, fluorescence of pHrodo Red SE was detected. The detailed detection conditions are as shown in the Scan Settings and Analysis Settings below.

Scan Settings

Scan Type: Non-Adherent Cell-by-Cell

Vessel Type: 96-well Corning

Image Channels: Phase, Orange (Acquisition Time: 400 ms)

Objective: 20×

Images per Well: 9

Analysis Settings

Analysis Type: Basic Analyzer

Phase Channel

Segmentation: AI Confluence

Cleanup:

- Hole Fill (μm^2): 0.000
- Adjust Size (pixels): 0

Filters:

- Area (μm^2): 60.000 or more
- Eccentricity: not set

Orange Channel

Segmentation: Surface Fit

- Threshold (OCU): 5.000
- Edge Split: On
- Edge Sensitivity: 0

Cleanup:

- Hole Fill (μm^2): 0.000
- Adjust Size (pixels): 0

Filters:

- Area (μm^2): not set
- Eccentricity: not set
- Mean Intensity: not set
- Mean Intensity: not set

[0170] FIG. 1 is a diagram showing the fluorescence detection area 22 hours after addition of LC-Plasma. FIG. 2 is a diagram showing the measurement results of IFN-α concentration in the culture supernatant collected 22 hours after addition of LC-Plasma. According to the results of FIG. 1, the amount of phagocytosis in the LC-Plasma+Cyt_D group was significantly smaller than that in the LC-Plasma+DMSO group. In addition, according to the results of FIG. 2, the IFN-α concentration in the LC-Plasma+Cyt_D group was significantly smaller than that in the LC-Plasma+DMSO group, and the activation of pDCs by LC-Plasma was significantly suppressed. From these results, it was elucidated that the activation of pDCs in which phagocytosis was inhibited was significantly suppressed.

[0171] <Example 2: Relationship between phagocytosis by pDCs and IFN-α production, in various lactic acid bacteria>

A cell suspension containing CAL-1 cells at 5.0×10^5 cells/mL was

prepared using serum-free medium, seeded at 10 mL/well in a 10 cm diameter culture dish, and cultured for 16 hours (pre-treatment step). CAL-1 cells were collected by pipetting. The collected CAL-1 cells were resuspended in serum-free medium at a concentration of 2.0×10^5 cells/mL and seeded at 200 μL/well in each well of a 96-well microplate. To the cell suspension in each of those wells, various lactic acid bacteria stained with pHrodo Red SE (Invitrogen, P36600) (ATCC 15577, ATCC 7963, ATCC 11955, JCM 1096, JCM 12533, JCM 9695, JCM 6125, or LC-Plasma) were added at a final concentration of 10 μg/mL. A negative group in which no bacteria were added was also prepared. Thereafter, the time-course measurement of fluorescence of pHrodo Red SE and the measurement of IFN-α concentration in the culture supernatant were performed in the same manner as in Example 1, except for the fluorescence intensity analysis conditions of the Incucyte(registered trademark) SX5 Live-Cell Analysis System (Sartorius). Note that the fluorescence intensity analysis conditions of the Incucyte(registered trademark) SX5 Live-Cell Analysis System (Sartorius) were as described in the following [Incucyte fluorescence intensity analysis 2]. The field of view area per image was 0.572 mm^2.

[0172] [Incucyte fluorescence intensity analysis 2]

In the Incucyte fluorescence intensity analysis, two channels were set: “Phase channel” and “Orange channel.” In the Phase channel, CAL-1 cells were detected based on phase contrast. In the Orange channel, fluorescence of pHrodo Red SE was detected. The detailed detection conditions are as shown in the Scan Settings and Analysis Settings below.

Scan Settings

Scan Type: Non-Adherent Cell-by-Cell

Vessel Type: 96-well Corning

Image Channels: Phase, Orange (Acquisition Time: 400 ms)

Objective: 20×

Images per Well: 9

Analysis Settings

Analysis Type: Basic Analyzer

Phase Channel

Segmentation: AI Confluence

Cleanup:

- Hole Fill (μm^2): 0.000
- Adjust Size (pixels): 0

Filters:

- Area (μm^2): 60.000 or more
- Eccentricity: not set

Orange Channel

Segmentation: Surface Fit

- Threshold (OCU): 5.000
- Edge Split: On
- Edge Sensitivity: 0

Cleanup:

- Hole Fill (μm^2): 0.000
- Adjust Size (pixels): 0

Filters:

- Area (μm^2): 20

- Eccentricity: not set
- Mean Intensity: not set
- Mean Intensity: not set

[0173] FIG. 3 is a diagram showing the fluorescence detection area 22 hours after addition of lactic acid bacteria for the eight species of lactic acid bacteria tested. FIG. 4 is a diagram showing the measurement results of IFN-α concentration in the culture supernatant collected 22 hours after addition of lactic acid bacteria for the 13 species of lactic acid bacteria tested. FIG. 5 is a diagram showing the correlation between the fluorescence detection area on the horizontal axis and the IFN-α concentration on the vertical axis for the eight species of lactic acid bacteria tested. According to the results of FIGs. 3 to 5, a strong positive correlation (R^2=0.91) was observed between the phagocytic behavior of each lactic acid bacterium and its immunostimulatory ability. From this, it was revealed that the immunostimulatory ability of bacteria is regulated by the amount of phagocytosis.

[0174] <Example 3: Relationship between phagocytosis of LC-Plasma via binding to BDCA2 by pDCs and IFN-α production>

To the CAL-1 cell suspension in each well seeded in the same manner as in Example 1, in the LC-Plasma+anti-BDCA2 group, an anti-BDCA2 antibody (Miltenyi Biotec, 130-090-690) was added at 10 μg/mL, and in the LC-Plasma+Isotype group, an isotype control of the same isotype and subclass as the anti-BDCA2 antibody (Miltenyi Biotec, 130-106-545) was added at 10 μg/mL to evaluate the effect of non-specific binding by the anti-BDCA2 antibody, followed by incubation for 30 minutes. In the LC-Plasma group as a control group, no antibody was

added. Thereafter, for each of the LC-Plasma group, LC-Plasma+Isotype group, and LC-Plasma+anti-BDCA2 group, exposure to LC-Plasma stained with pHrodo Red SE, phagocytosis evaluation, and measurement of IFN-α concentration in the culture supernatant were performed in the same manner as in Example 2.

[0175] FIG. 6 is a diagram showing the fluorescence detection area 22 hours after addition of LC-Plasma for the LC-Plasma group, LC-Plasma+Isotype group, and LC-Plasma+anti-BDCA2 group. FIG. 7 is a diagram showing the measurement results of IFN-α concentration in the culture supernatant collected 22 hours after addition of LC-Plasma for the LC-Plasma group, LC-Plasma+Isotype group, and LC-Plasma+anti-BDCA2 group. According to the results of FIGs. 6 and 7, in the LC-Plasma+anti-BDCA2 group in which the binding of BDCA2 to CAL-1 cells was inhibited by blocking with the anti-BDCA2 antibody, the amount of phagocytosis and the IFN-α concentration were smaller compared to the LC-Plasma group and LC-Plasma+Isotype group in which such binding was not inhibited. From these results, it was revealed that the binding of LC-Plasma to BDCA on the surface of pDCs is one of the stages that regulates the degree of immune activation by LC-Plasma. In addition, from these results, it was revealed that phagocytosis of LC-Plasma by pDCs via binding to BDCA is one of the stages that regulates the degree of immune activation by LC-Plasma.

[0176] <Example 4: Relationship between phagocytosis of various lactic acid bacteria via binding to BDCA2 by pDCs and IFN-α production>

To the CAL-1 cell suspension in each well seeded in the same manner as

in Example 1, in the anti-BDCA2 group, an anti-BDCA2 antibody (Miltenyi Biotec, 130-090-690) was added at 10 μg/mL, and in the Isotype group, its isotype control antibody (Miltenyi Biotec, 130-106-545) was added at 10 μg/mL, followed by incubation for 30 minutes. Thereafter, for each of the Isotype group and anti-BDCA2 group, exposure to the same various lactic acid bacteria tested in Example 2 stained with pHrodo Red SE, phagocytosis evaluation, and measurement of IFN-α concentration in the culture supernatant were performed in the same manner as in Example 2. Note that the amount of phagocytosis via binding to BDCA2 was calculated by the method shown below as the "Method for calculating the amount of phagocytosis via binding to BDCA2."

[0177] [Method for calculating the amount of phagocytosis via binding to BDCA2]

First, in order to take into account the effect of non-specific binding by the anti-BDCA2 antibody, the amount of phagocytosis of bacteria by pDCs in the Isotype group treated with an isotype control antibody having the same isotype and subclass as the anti-BDCA2 antibody (phagocytosis amount P) was evaluated. Next, the amount of phagocytosis of bacteria by pDCs in the anti-BDCA2 group treated with an anti-BDCA2 antibody that inhibits the binding of bacteria to BDCA2 (phagocytosis amount Q) was evaluated. Then, the value obtained by subtracting phagocytosis amount Q from phagocytosis amount P was calculated as the amount of phagocytosis via binding to BDCA2 by pDCs.

[0178] FIG. 8 is a diagram showing the fluorescence detection area 22

hours after addition of various lactic acid bacteria for the Isotype group and anti-BDCA2 group. FIG. 9 is a diagram showing the measurement results of IFN-α concentration in the culture supernatant collected 22 hours after addition of various lactic acid bacteria for the Isotype group and anti-BDCA2 group. FIG. 10 is a diagram showing the correlation between the amount of phagocytosis via binding to BDCA2 calculated according to the above method for calculating the amount of phagocytosis via binding to BDCA2 on the horizontal axis and the IFN-α concentration on the vertical axis for various lactic acid bacteria.

[0179] According to the results of FIGs. 8 and 9, in the anti-BDCA2 group in which the binding of BDCA2 to CAL-1 cells was inhibited by blocking with the anti-BDCA2 antibody, the amount of phagocytosis and the IFN-α concentration were smaller compared to the Isotype group in which such binding was not inhibited. From these results, it was revealed that, in bacteria other than LC-Plasma as well, the binding of bacteria to BDCA on the surface of pDCs is one of the stages that regulate the degree of immune activation by bacteria. In addition, from these results, it was revealed that phagocytosis of bacteria by pDCs via binding to BDCA is one of the stages that regulate the degree of immune activation by bacteria.

[0180] According to the results of FIG. 10, a strong positive correlation (R^2=0.86) was observed between the amount of phagocytosis via binding to BDCA2 of each lactic acid bacterium and its immunostimulatory ability. From this, it was shown not only that the immunostimulatory ability of bacteria is regulated by the amount of phagocytosis, but also that one of the major pathways of immune activation by bacteria is via

phagocytosis by pDCs via binding to BDCA2, and it was suggested that the binding ability to BDCA2 is practical as a parameter for evaluating the immunostimulatory ability of bacteria.

[0181] <Example 5: Construction of an assay for evaluating the binding ability of bacteria to BDCA2>

An assay for evaluating the binding ability of bacteria to BDCA2 (BDCA2 binding assay) was constructed with the protocol shown below. 100 μL of phosphate-buffered saline (PBS, Nacalai Tesque, 11482-15) in which $CaCl_2$ was dissolved at 0.5 mM (hereinafter also simply referred to as "buffer") was added to each well of a low-adsorption 96-well plate (Corning, 3474). To each of those wells, Recombinant Human BDCA-2 Fc Chimera Protein (R&D Systems, 1376-DL, hereinafter also simply referred to as "BDCA2") or Recombinant Human IgG1 Fc Protein (R&D Systems, 110-HG) was added at a final concentration of 1 to 50 μg/mL. Thereafter, LC-Plasma stained with FITC according to conventional methods to distinguish bacteria from contaminants was added to each of those wells at a final concentration of 10 μg/mL, and incubated at room temperature for 1 hour under conditions shielded from light with aluminum foil. After adding 150 μL of buffer to each of those wells, the 96-well plate was centrifuged at 400 g for 5 minutes at room temperature, and the supernatant was discarded. To each well, 100 μL of a solution prepared by diluting PE-conjugated $F(ab')_2$-Goat anti-Human IgG Fc Secondary Antibody (Thermo Fisher Scientific, H10104) 100-fold with buffer (hereinafter also referred to as "secondary antibody dilution") was added and incubated at room temperature for 15 minutes. The solution in each of those wells was subjected to flow cytometry

(FACS) using a flow cytometer (BD Biosciences, BD LSRFortessa(registered trademark) X-20 flow cytometer).

[0182] The analysis of FACS results was performed according to the following procedure. First, a population containing lactic acid bacteria was gated using FSC/SSC. Next, gating was performed at the wavelength corresponding to SSC/FITC, and only the FITC-stained population (FITC+ population) was extracted. For the FITC+ population, gating was performed at the wavelengths corresponding to SSC/PE, and the proportion of the population that fluoresced at the wavelength corresponding to PE (PE+ population) was extracted. Note that the threshold for the PE+ population was set so that the PE+ population was approximately 0.1% in the group to which huIgFc at the same concentration as each BDCA2 was added. The proportion of the PE+ population among the FITC+ population was calculated as the proportion of lactic acid bacteria bound to BDCA2.

[0183] FIG. 11 is a diagram showing the relative values when the proportion of LC-Plasma bound to BDCA2 at a BDCA2 concentration of 1 μg/mL is taken as 1, under conditions where the BDCA2 concentration is 1, 5, 10, 20, or 50 μg/mL relative to LC-Plasma at 10 μg/mL, as evaluated by BDCA2 binding assay. According to FIG. 11, the proportion of LC-Plasma bound to BDCA2 increased in a BDCA2 concentration-dependent manner. From this, it was revealed that the binding ability of bacteria to BDCA2 can be evaluated by the BDCA2 binding assay.

[0184] <Example 6: Phagocytosis of bacteria by pDCs, phagocytosis via binding to BDCA2, and binding ability to BDCA2>

The amount of phagocytosis by pDCs and the amount of phagocytosis via binding to BDCA2 by pDCs were evaluated for the lactic acid bacteria described in Tables 3 and 4, and the binding ability to BDCA2 was evaluated for the lactic acid bacteria described in Table 5. The amount of phagocytosis by pDCs was evaluated according to the same method as in Example 2, and is shown in Table 3 as (A-1) the fluorescence detection area 22 hours after addition of lactic acid bacteria. In addition, (A-2) the value obtained by subtracting the fluorescence detection area 22 hours after addition of *Lactococcus lactis* subsp. *lactis* ATCC 15577 from those fluorescence detection areas, and (A-3) the value (ratio) obtained by dividing those fluorescence detection areas by the fluorescence detection area 22 hours after addition of *Lactococcus lactis* subsp. *lactis* ATCC 15577 are shown in Table 4. The amount of phagocytosis via binding to BDCA2 by pDCs was evaluated according to the same method as in Example 4, and the results are shown in Table 3 as (B-1). In addition, the value obtained by subtracting the phagocytosis amount of *Lactococcus lactis* subsp. *lactis* ATCC 15577 from those phagocytosis amounts is shown as (B-2) in Table 4, and the value (ratio) obtained by dividing those phagocytosis amounts by the phagocytosis amount of *Lactococcus lactis* subsp. *lactis* ATCC 15577 is shown as (B-3) in Table 4. The binding ability to BDCA2 was evaluated by the same BDCA2 binding assay as in Example 5. Specifically, BDCA2 or Recombinant Human IgG1 Fc Protein (R&D Systems, 110-HG) was used at a final concentration of 10 μg/mL, and the test was performed under conditions where the lactic acid bacterium concentration was 10 μg/mL, and the proportion of lactic acid bacteria

bound to BDCA2 was measured as an indicator. Table 5 shows the proportion bound to BDCA2 as (C-1), the value obtained by subtracting the proportion of *Lactococcus lactis* subsp. *lactis* ATCC 15577 from those proportions as (C-2), and the value (ratio) obtained by dividing those proportions by the proportion of *Lactococcus lactis* subsp. *lactis* ATCC 15577 as (C-3). Note that since these measurements were performed over 3 or 4 days, the relative values shown as (A-2), (A-3), (B-2), (B-3), (C-2), and (C-3) relative to the results of *Lactococcus lactis* subsp. *lactis* ATCC 15577 (results of (A-1), (B-1), or (C-1)) were based on the values of *Lactococcus lactis* subsp. *lactis* ATCC 15577 tested on each measurement day as the basis for subtraction or division. In addition, for (A-2) the value obtained by subtracting the fluorescence detection area 22 hours after addition of *Lactococcus lactis* subsp. *lactis* ATCC 15577 from the fluorescence detection area 22 hours after addition of each lactic acid bacterium, and (B-2) the value obtained by subtracting the amount of phagocytosis via binding to BDCA2 of *Lactococcus lactis* subsp. *lactis* ATCC 15577 from the amount of phagocytosis via binding to BDCA2 of each lactic acid bacterium, values of 0 or less are shown as 0.

[0185] [Table 3]

Test day	Bacterial name	(A-1) Fluorescence detection area 22 hours after addition of each lactic acid bacterium (μm^2/image)	(B-1) Amount of phagocytosis via binding to BDCA2 of each lactic acid bacterium (μm^2/image)
Test 1	ATCC 15577	41.0	7.2
	ATCC 7962	3539.5	971.4
	ATCC 7963	701.3	406.8
	ATCC 11007	315.6	138.2
	ATCC 11454	1497.9	1349.2
	ATCC 11955	802.5	731.3
	ATCC 12929	705.0	402.6
	ATCC 13675	1741.2	905.5
	ATCC 15346	138.0	73.7
Test 2	ATCC 15577	49.7	5.9
	LC-Plasma	979.5	1067.2
	JCM 11056	1082.3	1260.5
	JCM 1096	311.2	219.7
	JCM 6985	0.0	13.0
	JCM 1717	97.3	80.0
	JCM 8573	273.9	3.3
Test 3	ATCC 15577	33.2	37.2
	LC-Plasma	1680.2	1216.6
	JCM 12533	96.1	153.9
	JCM 9695	443.4	438.2
	JCM 9698	15.3	22.8
	JCM 5707	579.6	108.3
	JCM 30200	71.3	77.3
	JCM 6125	206.3	74.7
	JCM 9890	278.2	42.5
	JCM 1174	68.3	22.7
	JCM 1190	313.7	196.9
	JCM 10602	261.2	0.0
	JCM 1255	102.2	13.4
	JCM 1217	4.5	0.0
	JCM 1222	57.8	0.0
	JCM 15461	4.4	27.9
	JCM 1205	88.0	20.7
	JCM 17295	24.9	0.0
Test 4	ATCC 15577	111.4	0.0
	CRL1505	260.3	229.4

[0186] [Table 4]

Test day	Bacterial name	(A-2)	(A-3)	(B-2)	(B-3)
Test 1	ATCC 15577	0.0	1.0	0.0	1.0
	ATCC 7962	3498.5	86.4	964.2	135.6
	ATCC 7963	660.3	17.1	399.6	56.8
	ATCC 11007	274.6	7.7	131.0	19.3
	ATCC 11454	1456.9	36.6	1342.1	188.3
	ATCC 11955	761.5	19.6	724.1	102.1
	ATCC 12929	664.1	17.2	395.4	56.2
	ATCC 13675	1700.2	42.5	898.3	126.4
	ATCC 15346	97.0	3.4	66.5	10.3
Test 2	ATCC 15577	0.0	1.0	0.0	1.0
	LC-Plasma	940.8	19.7	1061.3	180.9
	JCM 11056	1032.7	21.8	1254.6	213.6
	JCM 1096	261.5	6.3	213.8	37.2
	JCM 6985	0	0	7.1	2.2
	JCM 1717	47.7	2	74.1	13.6
	JCM 8573	224.2	5.5	0	0.6
Test 3	ATCC 15577	0.0	1.0	0.0	1.0
	LC-Plasma	1647.0	50.6	1179.4	32.7
	JCM 12533	63.0	2.9	116.8	4.1
	JCM 9695	410.2	13.4	401.0	11.8
	JCM 9698	0.0	0.5	0.0	0.6
	JCM 5707	546.4	17.5	71.1	2.9
	JCM 30200	38.2	2.2	40.1	2.1
	JCM 6125	173.1	6.2	37.6	2.0
	JCM 9890	245.0	8.4	5.4	1.1
	JCM 1174	35.1	2.1	0.0	0.6
	JCM 1190	280.5	9.5	159.7	5.3
	JCM 10602	228.1	7.9	0.0	0.0
	JCM 1255	69.1	3.1	0.0	0.4
	JCM 1217	0.0	0.1	0.0	0.0
	JCM 1222	24.7	1.7	0.0	0.0
	JCM 15461	0.0	0.1	0.0	0.8
	JCM 1205	54.8	2.7	0.0	0.6
	JCM 17295	0.0	0.7	0.0	0.0
Test 4	ATCC 15577	0.0	1.0	0.0	1.0
	CRL1505	148.9	2.3	229.4	N.D.

[0187] [Table 5]

Test day	Bacterial name	(C-1)	(C-2)	(C-3)
Test 1	ATCC 15577	3.0	0.0	1.0
	ATCC 15346	7.8	4.8	2.6
	ATCC 11007	15.1	12.1	5.0
	ATCC 11955	8.2	5.1	2.7
	ATCC 7962	47.0	44.0	15.7
Test 2	LC-Plasma	10.7	9.0	6.5
	ATCC 15577	1.6	0.0	1.0
	JCM 1174	20.1	18.5	12.3
	JCM 1217	84.1	82.4	51.3
	JCM 1222	2.3	0.6	1.4
	JCM 15461	2.8	1.2	1.7
Test 3	ATCC 15577	1.4	0.0	1.0
	CRL1505	3.4	2.0	2.4
	JCM 11056	3.3	1.9	2.3
	JCM 1096	56.0	54.6	39.2
	JCM 12533	51.0	49.6	35.7
	JCM 9695	10.0	8.6	7.0
	JCM 9698	2.5	1.1	1.8
	JCM 6125	26.8	25.4	18.7
	JCM 9890	8.3	6.8	5.8

[0188] Lactic acid bacteria in which the amount of phagocytosis by pDCs, the amount of phagocytosis via binding to BDCA2 by pDCs, and/or the binding ability to BDCA2 is at least higher than that of *Lactococcus lactis* subsp. *lactis* ATCC 15577 can be said to have immunostimulatory ability. Therefore, according to the results of Tables 3 to 5, lactic acid bacteria having a higher amount of phagocytosis by pDCs, amount of phagocytosis via binding to BDCA2 by pDCs, and/or binding ability to BDCA2 than those of *Lactococcus lactis* subsp. *lactis* ATCC 15577 can be said to have immunostimulatory ability.

[0189] <Example 7: Importance of phagocytosis in IFN-α production by pDCs>

To the CAL-1 cell suspension in each well seeded in the same manner as

in Example 1, in the group to which cytochalasin D (described as CytD in the graph) was added, 400 μM cytochalasin D (Fujifilm Wako, 037-17561) dissolved in 4% by volume dimethyl sulfoxide (DMSO) was added so that cytochalasin D and DMSO were at final concentrations of 10 μM and 0.1% by volume, respectively, followed by incubation for 30 minutes. As a control, in the DMSO group, only 4% by volume DMSO was added at a final concentration of 0.1% by volume, followed by incubation for 30 minutes in the same manner. To each well after incubation, the lactic acid bacteria described in Table 1 stained with pHrodo Red SE (Invitrogen, P36600) by the method of the above-described [Staining of bacteria with pHrodo Red SE] were added at a final concentration of 10 μg/mL, followed by culturing for 24 hours. The phagocytic activity and IFN-α concentration in each well were measured in the same manner as in Example 1.

[0190] In addition, to confirm that DMSO and cytochalasin D had no cytotoxicity, cytochalasin D or DMSO, or PBS of the same volume as a control thereof, was added in the same manner as described above, followed by incubation for 30 minutes and then culturing for 24 hours. The cytotoxicity at that time was evaluated by measuring the number of viable cells using Cell Counting Kit-8 (Dojindo, CK04).

[0191] Cell Counting Kit-8 is a kit for measuring cell number in cell proliferation or chemical sensitivity tests. By adopting the novel tetrazolium salt WST-8, which produces highly sensitive water-soluble formazan, as a chromogenic substrate, higher sensitivity measurement than the conventional Cell Counting Kit has become possible. WST-8 is reduced by intracellular dehydrogenases to produce water-soluble

formazan. By directly measuring the absorbance of this formazan at 450 nm, the number of viable cells can be easily counted. The amount of formazan produced is in a linear proportional relationship with the number of cells. Cytotoxicity can be evaluated by observing changes in the number of viable cells.

[0192] In addition, as described in detail in Example 1, cytochalasin D is an actin polymerization inhibitor, and it is known that phagocytosis is suppressed in cells to which cytochalasin D has been added.

[0193] FIG. 12 is a diagram showing the results of measuring the number of viable cells by Cell Counting Kit-8 after addition of DMSO or cytochalasin D+DMSO (described as CytD in the graph) and 24 hours of culture. FIG. 13 is a diagram showing the Total Integrate Intensity (TII, OCU×μm^2/Image), which is an indicator of phagocytic activity representing the total amount of fluorescence, 24 hours after addition of lactic acid bacteria for the six species of lactic acid bacteria tested. FIG. 14 is a diagram showing the IFN-α concentration in the supernatant 24 hours after addition of lactic acid bacteria for the six species of lactic acid bacteria tested. According to the results of FIG. 12, the addition of DMSO or cytochalasin D showed no change in the number of viable cells compared to the group to which PBS was added, and this result indicates that the inhibitory reagents used in this test do not cause cytotoxicity. According to the results of FIG. 13, the phagocytic activity of various lactic acid bacteria was completely suppressed by treatment with cytochalasin D. At this time, according to the results of FIG. 14, IFN-α production completely disappeared by inhibiting phagocytic activity. From this, it was shown that phagocytosis is essential for

immune activation of pDCs by lactic acid bacteria, and considering the results of Example 1 as well, it was shown that phagocytosis regulates immune activation of pDCs.

[0194] <Example 8: Measurement of phagocytosis ratio using FACS>

Using the same protocol as in Example 2, various lactic acid bacteria stained with pHrodo Red SE (ATCC 15577, ATCC 7963, ATCC 11955, JCM 1096, JCM 12533, JCM 9695, ATCC 7962, JCM 1185, JCM 1149, or LC-Plasma) were added to CAL-1 cells. After incubating at 37°C for 24 hours, cells were collected using FACS buffer, and 7-AAD (manufacturer: BD Biosciences, BD LSRFortessa) staining was performed. After washing the cells with FACS buffer, the washed cells were subjected to flow cytometry (FACS) using a flow cytometer (BD Biosciences, BD LSRFortessa(registered trademark) X-20 flow cytometer). The IFN-α in the supernatant when the cells were collected was evaluated in the same manner as in Example 1.

[0195] The analysis of FACS results was performed according to the following procedure. First, a cluster containing CAL-1 cells was gated using FSC/SSC. Next, gating was performed at the wavelength corresponding to SSC/7-AAD, and only the population not stained with 7-AAD (7-AAD- population: population of living CAL-1 cells) was extracted. For the 7-AAD- population, gating was performed at the wavelength corresponding to PE/SSC, and the proportion of the population in which fluorescence at the wavelength corresponding to PE (i.e., fluorescence derived from pHrodo Red SE that labels lactic acid) was detected (PE+ population) was extracted. The proportion of the PE+ population relative to the 7-AAD- population was taken as the

phagocytosis ratio. Note that the threshold for the PE+ population was set to the minimum value of the population with stronger PE intensity among the two populations that separated when PE/SSC was plotted.

[0196] Table 6 is a table summarizing the results of measuring the phagocytosis ratio and IFN-α of various lactic acid bacteria. According to the results of Table 6, it was confirmed that the phagocytosis ratio obtained by flow cytometer and IFN-α have a positive correlation. A positive correlation (R^2=0.69) was observed between the phagocytic behavior of each lactic acid bacterium and its immunostimulatory ability. From this result, it was revealed that the immunostimulatory ability of bacteria is regulated by the amount of phagocytosis.

[Table 6]

Strain name	IFN-α(pg/mL)	phagocytosis ratio
LC-Plasma	1365.3	12.3%
ATCC 15577	3.6	2.4%
ATCC 7963	470.8	6.6%
ATCC 11955	408.7	9.4%
JCM 1096	348.5	7.4%
JCM 12533	20.6	2.4%
JCM 9695	365.2	9.5%
ATCC 7962	1746.7	12.7%
JCM 1185	204.6	5.3%
JCM 1149	478.3	10.3%

[0197] <Example 9: Acquisition of lactic acid bacterium strains with high phagocytosis ratio>

In the present Example, LC-Plasma was cultured in two different types of media, and the phagocytosis ratio and IFN-α were evaluated. One of the two types of LC-Plasma cultured in different media is LC-Plasma cultured according to Condition B in Table 1. The other was cultured

according to the following method. The bacterial cells were suspended in MRS medium (product name: Difco Lactobacilli MRS Broth, manufacturer: Becton, Dickinson and Company; the same applies hereinafter) and seeded on MRS agar medium. After culturing at 30℃ for 48 hours, colony picking was performed, and the cells were seeded in 10 mL of M17 medium (product name: M17 Broth, manufacturer: Becton, Dickinson and Company) supplemented with glucose (product name: D(+)-Glucose, manufacturer: Fujifilm Wako Pure Chemical) at a final concentration of 1%, and cultured at 30℃ for 24 hours. 1 mL of the cultured bacterial suspension was seeded in 100 mL of M17 medium and cultured at 30℃ for 24 hours.

[0198] The phagocytosis ratio and IFN-α were evaluated for LC-Plasma cultured in the two types of media in the same manner as in Example 8.

[0199] Table 7 is a table summarizing the results of measuring the phagocytosis ratio and IFN-α of the two strains. According to the results of Table 7, LC-Plasma cultured in M17 medium supplemented with 1% glucose had a higher phagocytosis ratio than LC-Plasma cultured in MRS medium, and lactic acid bacteria with enhanced immunostimulatory ability can be obtained.

[Table 7]

Strain name	Medium	IFNα (pg/mL)	phagocytosis ratio
LC-Plasma	MRS medium	1365.3	12.3%
LC-Plasma	M17 medium + 1% glucose	1531.9	17.9%

CLAIMS

[Claim 1] An immunostimulatory composition comprising, as an active ingredient, a bacterium that binds to blood dendritic cell antigen 2 (BDCA2).

[Claim 2] The composition according to claim 1, wherein the binding ability of the bacterium to BDCA2 is greater than the binding ability of *Lactococcus lactis* subsp. *lactis* ATCC 15577 to BDCA2.

[Claim 3] The composition according to claim 2, wherein the binding ability to BDCA2 is evaluated based on the proportion of bacteria bound to BDCA2 as evaluated in a binding assay.

[Claim 4] An immunostimulatory composition comprising, as an active ingredient, a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC), wherein the bacterium satisfies the following Requirement X:

[Requirement X] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the bacterium at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the bacterium to the total number of the pDCs brought into contact is 2.5% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[Claim 5] An immunostimulatory composition comprising, as an active ingredient, a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC),

wherein the amount of phagocytosis of the bacterium by the pDC is 2.0 times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 by the pDC.

[Claim 6] A composition comprising, as a bacterium, *Lactococcus lactis* subsp. *lactis* JCM 5805, wherein the *Lactococcus lactis* subsp. *lactis* JCM 5805 satisfies the following Requirement Y:

[Requirement Y] after pDCs at a final concentration of 2.0×10^5 cells/mL are brought into contact with the *Lactococcus lactis* subsp. *lactis* JCM 5805 at a final concentration of 10 μg/mL in a serum-free medium for 24 hours, the ratio of the number of the pDCs that have phagocytosed the *Lactococcus lactis* subsp. *lactis* JCM 5805 to the total number of the pDCs brought into contact is 15.0% or more, provided that the pDCs are pDCs pre-cultured in a serum-free medium.

[Claim 7] The composition according to claim 6, wherein the composition is an immunostimulatory composition.

[Claim 8] The composition according to any one of claims 1 to 5, wherein the bacterium is a lactic acid bacterium and/or an acetic acid bacterium.

[Claim 9] The composition according to any one of claims 1 to 5, wherein the bacterium is one or two or more bacteria selected from the group consisting of bacteria of the genus *Lactococcus*, bacteria of the genus *Leuconostoc*, bacteria of the genus *Streptococcus*, bacteria of the genus *Enterococcus*, bacteria of the genus *Tetragenococcus*, bacteria of the genus *Oenococcus*, bacteria of the genus *Weissella*, bacteria of the genus *Bifidobacterium*, and bacteria of the genus *Lactobacillus*.

[Claim 10] The composition according to any one of claims 1 to 5, wherein the bacterium is *Lactococcus lactis* subsp. *lactis*.

[Claim 11] The composition according to any one of claims 1 to 5, wherein the bacterium is one or two or more bacteria selected from the

group consisting of *Lactococcus plantarum* JCM 11056, *Lactococcus curvatus* JCM 1096, *Lactococcus murinus* JCM 1717, *Lactococcus parakefiri* JCM 8573, *Lactococcus paraplantarum* JCM 12533, *Leuconostoc carnosum* JCM 9695, *Streptococcus salivarius* JCM 5707, *Enterococcus lactis* JCM 30200, *Oenococcus oeni* JCM 6125, *Weissella paramesenteroides* JCM 9890, *Weissella viridescens* JCM 1174, *Bifidobacterium animalis* subsp. *animalis* JCM 1190, *Bifidobacterium animalis* subsp. *lactis* JCM 10602, *Bifidobacterium bifidum* JCM 1255, *Bifidobacterium longum* subsp. *infantis* JCM 1222, *Bifidobacterium pseudolongum* JCM 1205, *Lactococcus lactis* subsp. *lactis* ATCC 7963, *Lactococcus lactis* subsp. *lactis* ATCC 7962, *Lactococcus lactis* subsp. *lactis* ATCC 15346, *Lactococcus lactis* subsp. *lactis* ATCC 13675, *Lactococcus lactis* subsp. *lactis* ATCC 12929, *Lactococcus lactis* subsp. *lactis* ATCC 11955, *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactococcus lactis* subsp. *lactis* ATCC 11007, and *Lactobacillus rhamnosus* CRL1505.

[Claim 12] The composition according to any one of claims 1 to 7, wherein the bacterium comprises a dead bacterium.

[Claim 13] The composition according to any one of claims 1 to 7, wherein the bacterium is a bacterium that is phagocytosed by a plasmacytoid dendritic cell (pDC) via binding to blood dendritic cell antigen 2 (BDCA2).

[Claim 14] The composition according to claim 13, wherein the phagocytosis of the bacterium by the pDC includes phagocytosis via binding to BDCA2 and phagocytosis not via binding to BDCA2, and the amount of phagocytosis of the bacterium via binding to BDCA2 is 2.0

times or more the amount of phagocytosis of *Lactococcus lactis* subsp. *lactis* ATCC 15577 via binding to BDCA2.

[Claim 15] The composition according to claim 14, wherein the amount of phagocytosis not via binding to BDCA2 is the amount of phagocytosis of the bacterium by the pDC in a state where BDCA2 on the pDC cannot bind to a ligand.

[Claim 16] The composition according to claim 13, wherein the bacterium promotes production of IFN-α by the pDC via phagocytosis by the pDC.

[Claim 17] The composition according to any one of claims 1 to 7, which is a food composition, a pharmaceutical composition, or a feed.

[Claim 18] The composition according to claim 17, wherein the number of bacteria per unit package in the food composition, pharmaceutical composition, or feed is 1×10^8 or more.

[Claim 19] A method for screening a bacterium having immunostimulatory ability, comprising a step of screening the bacterium having the immunostimulatory ability using binding to blood dendritic cell antigen 2 (BDCA2) as an indicator.

[Claim 20] The method according to claim 19, wherein the screening step is performed by a binding assay between a candidate bacterium and BDCA2.

[Claim 21] A method for enhancing immunostimulatory ability and/or susceptibility to phagocytosis by a plasmacytoid dendritic cell (pDC) of a bacterium, comprising a step of culturing the bacterium in M17 medium supplemented with glucose at a final concentration of 0.1 to 10 mass%.

[Claim 22] A method for producing an immunostimulatory composition comprising *Lactococcus lactis* subsp. *lactis* JCM 5805 as an active ingredient, comprising a step of culturing *Lactococcus lactis* subsp. *lactis* JCM 5805 in M17 medium supplemented with glucose at a final concentration of 0.1 to 10 mass%.