

Claims

- [Claim 1] A method for producing a sulfated polysaccharide, the method comprising the following steps (1-1) and (1-2):
(1-1) preparing a transformant (a) of a bacterium of the genus *Corynebacterium*, comprising at least a gene encoding an ATP sulfurylase and a gene encoding an APS kinase which are introduced thereinto in an expressible manner, or a treated matter of the transformant (a); and
(1-2) conducting a reaction for producing PAPS by using a reaction solution containing ATP or an ATP source, a sulfate ion source, and the transformant (a) or the treated matter thereof.
- [Claim 2] The method for producing a sulfated polysaccharide according to claim 1, the method further comprising the following steps (2-1) and (2-2):
(2-1) preparing a transformant (b) of a microorganism belonging to prokaryotes, comprising at least a gene encoding a C5-epimerase which is introduced thereinto in an expressible manner, or a treated matter or extract of the transformant (b); and
(2-2) conducting C5-epimerization by incorporating the transformant (b) or the treated matter or extract thereof in the reaction solution in the presence of N-sulfoheparosan.
- [Claim 3] The method for producing a sulfated polysaccharide according to claim 1 or 2, comprising conducting sulfation by a transformant comprising a gene encoding sulfotransferase, which is introduced thereinto in an expressible manner, or a treated matter or extract of the transformant.
- [Claim 4] The method for producing a sulfated polysaccharide according to claim 3, the method further comprising the following steps (3-1) and (3-2):
(3-1) preparing a transformant (c) of a microorganism belonging to prokaryotes, comprising at least a gene encoding a 2-O-sulfotransferase which is introduced thereinto in an expressible manner, or a treated matter or extract of the transformant (c); and
(3-2) conducting 2-O-sulfation by incorporating the transformant (c) or the treated matter or extract thereof in the reaction solution in the presence of N-sulfoheparosan.
- [Claim 5] The method for producing a sulfated polysaccharide according to claim 3 or 4, the method further comprising the following steps (3'-1) to (3'-3):
(3'-1) preparing a transformant (b) of a microorganism belonging to

prokaryotes, comprising at least a gene encoding a C5-epimerase which is introduced therein in an expressible manner, or a treated matter or extract of the transformant (b);

(3'-2) preparing a transformant (c) of a microorganism belonging to prokaryotes, comprising at least a gene encoding a 2-O-sulfotransferase which is introduced therein in an expressible manner, or a treated matter or extract of the transformant (c); and

(3'-3) conducting C5-epimerization and 2-O-sulfation by incorporating the transformant (b) or the treated matter or extract thereof, and the transformant (c) or the treated matter or extract thereof in the reaction solution in the presence of N-sulfoheparosan.

[Claim 6]

The method for producing a sulfated polysaccharide according to any one of claims 3 to 5, the method further comprising the following steps (4-1) and (4-2):

(4-1) preparing a transformant (d) of a microorganism belonging to prokaryotes, comprising at least a gene encoding a 6-O-sulfotransferase which is introduced therein in an expressible manner, or a treated matter or extract of the transformant (d); and

(4-2) conducting 6-O-sulfation by incorporating the transformant (d) or the treated matter or extract thereof in the reaction solution in the presence of N-sulfoheparosan.

[Claim 7]

The method for producing a sulfated polysaccharide according to any one of claims 3 to 6, the method further comprising the following steps (5-1) and (5-2):

(5-1) preparing a transformant (e) of a microorganism belonging to prokaryotes, comprising at least a gene encoding a 3-O-sulfotransferase which is introduced therein in an expressible manner, or a treated matter or extract of the transformant (e); and

(5-2) conducting 3-O-sulfation by incorporating the transformant (e) or the treated matter or extract thereof in the reaction solution in the presence of N-sulfoheparosan.

[Claim 8]

A method for producing a sulfated polysaccharide, the method comprising generating a sulfated polysaccharide by incorporating, in a reaction solution in the presence of ATP or an ATP source, a sulfate ion source, and N-sulfoheparosan,

a transformant (a) of a bacterium of the genus *Corynebacterium*, comprising at least a gene encoding an ATP sulfurylase and a gene encoding an APS kinase which are introduced therein in an ex-

pressible manner, or a treated matter of the transformant (a), and at least one selected from:
a transformant (b) of a microorganism belonging to prokaryotes, comprising at least a gene encoding a C5-epimerase which is introduced thereinto in an expressible manner, or a treated matter or extract of the transformant (b),
a transformant (c) of a microorganism belonging to prokaryotes, comprising at least a gene encoding a 2-O-sulfotransferase which is introduced thereinto in an expressible manner, or a treated matter or extract of the transformant (c),
a transformant (d) of a microorganism belonging to prokaryotes, comprising at least a gene encoding a 6-O-sulfotransferase which is introduced thereinto in an expressible manner, or a treated matter or extract of the transformant (d), and
a transformant (e) of a microorganism belonging to prokaryotes, comprising at least a gene encoding a 3-O-sulfotransferase which is introduced thereinto in an expressible manner, or a treated matter or extract of the transformant (e).

[Claim 9] The method for producing a sulfated polysaccharide according to claim 8, the method further comprising generating a sulfated polysaccharide by incorporating, in the reaction solution in the presence of ATP or the ATP source, the sulfate ion source, and N-sulfoheparosan, the transformant (a) or the treated matter thereof, and the transformants (b) to (e) or the treated matters or extracts thereof.

[Claim 10] The method for producing a sulfated polysaccharide according to any one of claims 1 to 9, which is for producing heparin.

[Claim 11] A method for producing PAPS, the method comprising the following steps (i) and (ii):
(i) preparing a transformant of a bacterium of the genus *Corynebacterium*, comprising at least a gene encoding an ATP sulfurylase and a gene encoding an APS kinase which are introduced thereinto in an expressible manner, or a treated matter of the transformant; and
(ii) conducting a reaction for producing PAPS by using a reaction solution containing ATP or an ATP source, a sulfate ion source, and the transformant prepared in the step (i) or the treated matter thereof.