Patent Document 3: EP1227152A1

Patent Document 4: Japanese unexamined Patent Application Publication No. 2010-35472

Patent Document 5: Japanese unexamined Patent Application Publication No. 2011-87586

Patent Document 6: Japanese unexamined Patent Application Publication No. 2006-180708

Summary

Object to be Solved by the Invention

[0007]

It is an object of the present invention to provide signal sequence information capable of secreting an antibody to the outside of cells in generation of the antibody by bifidobacteria, an antibody expression vector capable of secreting an antibody to the outside of cells by utilizing the signal sequence information, and bifidobacteria which are transformed with the antibody expression vector and are capable of secreting an antibody.

Means to Solve the Object

[0008]

It has been well known that a HER2 gene product (a cell division-stimulating receptor HER2 that is present on a cell surface) is overproduced by gene amplification in breast cancer, stomach cancer, prostate cancer and the like, and thus that such overproduction becomes a cancer exacerbation factor. In antibody therapy, and particularly, in antibody therapy for cancer, it is important to allow an antibody to locally express and/or

secrete in cancer. Thus, the present inventors have used a plasmid for transformation of bifidobacteria having the signal sequence disclosed in the aforementioned Patent Document 1 or 2, with regard to a signal sequence that can be applied to a plasmid vector for transformation of bifidobacteria. As a result, it was found that secretion of antibodies is not sufficient. Hence, the inventors have studied novel signal sequences, and have found that Bifidobacterium longum transformed with a vector having inserted thereinto a DNA insert comprising a Trastuzumab single-chain antibody (scFv) gene linked to the 3'-terminus of a DNA encoding signal sequences named as SP27 and SP7 or of a signal peptide-linker conjugate having a linker sequence linked to such a signal sequence, efficiently secretes Trastuzumab to the outside of the cells, thereby completing the present disclosure.

[0008a]

In one aspect, the present disclosure refers to a DNA encoding a signal peptide-linker conjugate having a linker consisting of an amino acid sequence linked to the C-terminus of a signal peptide consisting of an amino acid sequence described in the following a) or b): a) an amino acid sequence shown in SEQ ID NO: 1 or SEQ ID NO: 107; or b) an amino acid sequence comprising a deletion, substitution or addition of one or two amino acids, with respect to the amino acid sequence shown in SEQ ID NO: 1 or SEQ ID NO: 107, wherein a peptide consisting of the amino acid sequence functions as a signal peptide in Bifidobacterium longum, and has a sequence identity of 95%

or more with the amino acid sequence before the deletion, substitution or addition.

[0008b]

In another aspect, the present disclosure refers to a DNA insert, wherein the 5'-terminus of an antibody gene is linked to the 3'-terminus of the DNA as described herein.

[0008c]

In yet another aspect, the present disclosure refers to a vector having the DNA insert as described herein inserted thereinto.

[0008d]

In yet another aspect, the present disclosure refers to an intestinal bacterium transformed with the vector as described herein.

[0008e]

In yet another aspect, the present disclosure refers to an antibody drug composition comprising, as an active ingredient, the intestinal bacterium as described herein.

[0009]

Specifically, the present disclosure relates to the following:

[1] A DNA encoding a signal peptide consisting of an amino acid sequence described in the following a) or b):

a) an amino acid sequence shown in SEQ ID NO: 1 (SP27) or SEQ ID NO: 107 (SP7); or

b) an amino acid sequence comprising a deletion, substitution or addition of one or several amino acids, with respect to the amino acid sequence shown in SEQ ID

NO: 1 or SEQ ID NO: 107, wherein a peptide consisting of the amino acid sequence functions as a signal peptide in Bifidobacterium longum,

[2] The DNA according to the above [1], consisting of a

CLAIMS

[Claim 1]

A DNA encoding a signal peptide-linker conjugate having a linker consisting of an amino acid sequence linked to the C-terminus of a signal peptide consisting of an amino acid sequence described in the following a) or b):

a) an amino acid sequence shown in SEQ ID NO: 1 or SEQ ID NO: 107; or

b) an amino acid sequence comprising a deletion, substitution or addition of one or two amino acids, with respect to the amino acid sequence shown in SEQ ID NO: 1 or SEQ ID NO: 107, wherein a peptide consisting of the amino acid sequence functions as a signal peptide in Bifidobacterium longum, and has a sequence identity of 95% or more with the amino acid sequence before the deletion, substitution or addition.

[Claim 2]

The DNA according to claim 1, wherein the signal peptide-linker conjugate consists of an amino acid sequence shown in SEQ ID NO: 3 or SEQ ID NO: 109.

[Claim 3]

The DNA according to claim 2, consisting of a nucleotide sequence shown in SEQ ID NO: 4 or SEQ ID NO: 110.

[Claim 4]

A DNA insert, wherein the 5'-terminus of an antibody gene is linked to the 3'-terminus of the DNA according to any one of claims 1 to 3.

[Claim 5]

The DNA insert according to claim 4, wherein the antibody gene is the gene of an antibody having an anticancer activity.

[Claim 6]

The DNA insert according to claim 5, wherein the antibody having an anticancer activity is Trastuzumab.

[Claim 7]

The DNA insert according to claim 6, wherein the Trastuzumab is a Trastuzumab single-chain antibody.

[Claim 8]

A vector having the DNA insert according to any one of claims 4 to 7 inserted thereinto.

[Claim 9]

An intestinal bacterium transformed with the vector according to claim 8.

[Claim 10]

The intestinal bacterium according to claim 9, wherein the bacterium is a microorganism belonging to genus Bifidobacterium.

[Claim 11]

The intestinal bacterium according to claim 10, wherein the microorganism belonging to genus Bifidobacterium is Bifidobacterium longum.

[Claim 12]

An antibody drug composition comprising, as an active ingredient, the intestinal bacterium according to any one of claims 9 to 11.

[Claim 13]

The antibody drug composition according to claim 12, which is an anticancer agent composition.