

[DESCRIPTION]

[Invention Title]

NOVEL PSICOSE-6-PHOSPHATE PHOSPHATASE, COMPOSITION FOR PRODUCING PSICOSE COMPRISING THE SAME, AND METHOD FOR PRODUCING PSICOSE USING THE SAME

[Technical Field]

The present application relates to a novel enzyme for dephosphorylating psicose-6-phosphate for the production of psicose, a composition for producing psicose including the same, and a method for producing psicose using the same.

[Background Art]

D-Psicose-3-epimerase (EC 5.1.3.30) and D-tagatose-3-epimerase (EC 5.1.3.31) are known as enzymes that catalyze the 3-epimerization of D-fructose to produce D-psicose. When D-psicose is produced through a single enzymatic reaction using the enzyme, reaction equilibrium between the substrate (*i.e.*, D-fructose) and the product (*i.e.*, D-psicose) exists at a constant level (product/substrate = appropriately 20–35%). Thus, the production of high-purity D-psicose requires an additional process for separating and removing a relatively high concentration of D-fructose from the enzymatic reaction product. Additionally, fructose is a relatively expensive raw material compared to starch or glucose, and when fructose is used as a raw material, the cost ratio of psicose and tagatose increases. Accordingly, various studies on the production of allulose and tagatose through a reaction using starch or glucose, which is relatively economical raw material, have been reported (KR 10-2018-0004023, WO 2018-112139, WO 2017-059278, WO 2018-129275).

Meanwhile, Chan *et al.* (2008. *Biochemistry*. 47:9608–9617) reported D-ribulose-5-phosphate-3-epimerase (EC 5.1.3.1) derived from *Streptococcus pyogenes* and D-psicose-6-phosphate-3-epimerase (EC 5.1.3.-) derived from *E. coli* capable of catalyzing the 3-epimerization of D-fructose-6-phosphate and D-psicose-6-phosphate. However, these enzymes are not industrially applicable due to poor thermal resistance.

[Disclosure]

[Technical Problem]

Under such circumstances, the present inventors have earnestly conducted research to develop a method for increasing the conversion rate of allulose on an industrial scale in an

economical manner. As a result, the present inventors have found that after sucrose or starch (*e.g.*, maltodextrin) as an inexpensive raw material is converted to psicose-6-phosphate, the use of the psicose-6-phosphate phosphatase of the present application, which is specific to psicose-6-phosphate and participates in an irreversible reaction pathway, enables the production of psicose through one-pot enzymatic conversions using a plurality of enzymes involved in the psicose production pathways and can significantly increase the rate of conversion to psicose. Accordingly, the present application has been accomplished based on this finding. The enzyme of the present application has better advantages in the production of psicose compared to the conventionally known psicose-6-phosphate phosphatase (Panoramic view of a superfamily of phosphatases through substrate profiling, *PNAS*, 06.04. 2015, Huang, *etc.*).

[Technical Solution]

It is one object of the present application to provide a psicose-6-phosphate phosphatase.

It is another object of the present application to provide a nucleic acid encoding the psicose-6-phosphate phosphatase.

It is still another object of the present application to provide a transformant including the nucleic acid encoding the psicose-6-phosphate phosphatase.

It is yet another object of the present application to provide a composition for producing psicose, including the psicose-6-phosphate phosphatase, a microorganism expressing the same, or a culture of the microorganism.

It is even another object of the present application to provide a method for producing psicose, including converting psicose-6-phosphate into psicose by bringing psicose-6-phosphate into contact with the psicose-6-phosphate phosphatase, a microorganism expressing the same, or a culture of the microorganism.

[Advantageous Effects]

The novel enzymes and combination thereof of the present application are thermoresistant, and accordingly, the enzyme of the present application can participate in the pathway for the conversion of psicose-6-phosphate to psicose in an industrial manner, allows the progression of the pathway for the synthesis of psicose from glucose or starch (*e.g.*, maltodextrin) as an inexpensive raw material, and enables the production of psicose through the dephosphorylation of psicose-6-phosphate, an irreversible reaction pathway, thereby significantly increasing the rate of conversion to psicose. Thermoresistant enzymes can commercially prevent microorganisms and easily react with high-concentration substrates, and

thus their effects are prominent. In addition, the production method of the present application can simplify or eliminate the separation and purification process, including the high concentration of the reaction product (*i.e.*, psicose) by the increase in the conversion rate of psicose, and thus there is an economic advantage, while allowing a simple production method. In particular, separation using SMB can be minimized or eliminated, thus maximizing separation efficiency and yield.

[Detailed Description of Embodiments]

Hereinafter, the present application will be described in detail. Meanwhile, the description of one aspect and embodiment disclosed in the present application may also be applied to other aspects and embodiments with respect to common elements. Moreover, all combinations of various elements disclosed in the present application fall within the scope of the present application. In addition, it does not appear that the scope of the present application is limited by the following detailed description.

In order to achieve the objects above, one aspect of the present application provides a psicose-6-phosphate phosphatase.

Specifically, the psicose-6-phosphate phosphatase of the present application may be a psicose-6-phosphate phosphatase derived from the genus of *Alicyclobacillus*, *Amycolatopsis*, *Anaerolinea*, *Archaeoglobus*, *Bacillus*, *Caldicellulosiruptor*, *Caldilinea*, *Caldithrix*, *Carboxydocella*, *Carboxydotherrmus*, *Chloroflexi*, *Defluviitoga*, *Deinococcus*, *Desulfurococcus*, *Dictyoglomus*, *Effusibacillus*, *Fervidobacterium*, *Geobacillus*, *Halococcus*, *Hydrogenivirga*, *Hydrogenobacter*, *Hyperthermus*, *Kosmotoga*, *Marinitoga*, *Meiothermus*, *Mesotoga*, *Metallosphaera*, *Methanocella*, *Methanococcoides*, *Methanohalobium*, *Methanolobus*, *Methanosarcina*, *Methanothermus*, *Petrotoga*, *Picrophilus*, *Pseudonocardia*, *Pyrococcus*, *Pyrodictium*, *Rhodothermus*, *Slackia*, *Staphylothermus*, *Sulfolobus*, *Thermanaerotherrix*, *Thermoanaerobacter*, *Thermoanaerobacterium*, *Thermobifida*, *Thermococcus*, *Thermocrinis*, *Thermoflexus*, *Thermotoga*, *Thermus*, and *Truepera*, and more specifically derived from *Alicyclobacillus acidocaldarius*, *Alicyclobacillus tengchongensis*, *Amycolatopsis thermoflava*, *Anaerolinea thermolimosa*, *Anaerolinea thermophila*, *Archaeoglobus fugidus*, *Archaeoglobus profundus*, *Archaeoglobus veneficus*, *Bacillus licheniformis*, *Caldicellulosiruptor bescii*, *Caldilinea aerophila*, *Caldithrix abyssi*, *Carboxydocella* sp. ULO1, *Carboxydotherrmus ferrireducens*, *Chloroflexi bacterium* 54-19, *Defluviitoga tunisiensis*, *Deinococcus aerius*, *Deinococcus apachensis*, *Deinococcus aquatilis*, *Deinococcus geothermalis*, *Deinococcus*

hopiensis, *Deinococcus maricopensis*, *Deinococcus murrayi*, *Deinococcus reticulitermitis*, *Deinococcus wulumuqiensis*, *Deinococcus* sp. Leaf326, *Deinococcus phoenicis*, *Deinococcus proteolyticus*, *Deinococcus* sp. 17bor-2, *Deinococcus* sp. NW-56, *Deinococcus* sp. RL, *Deinococcus* sp. YIM 77859, *Desulfurococcus mucosus*, *Dictyoglomus turgidum*, *Effusibacillus pohliae*, *Fervidobacterium gondwanense*, *Fervidobacterium islandicum*, *Fervidobacterium nodosum*, *Fervidobacterium pennivorans*, *Geobacillus* sp., *Geobacillus stearothermophilus*, *Halococcus salifodinae*, *Hydrogenivirga* sp. 128-5-R1-1, *Hydrogenobacter hydrogenophilus*, *Hydrogenobacter thermophilus*, *Hyperthermus butylicus*, *Kosmotoga arenicorallina*, *Kosmotoga olearia*, *Marinitoga piezophila*, *Meiothermus cerbereus*, *Meiothermus chliarophilus*, *Meiothermus ruber*, *Meiothermus Silvanus*, *Meiothermus taiwanensis*, *Meiothermus timidus*, *Meiothermus rufus*, *Mesotoga infera*, *Metallosphaera sedula*, *Methanocella conradii*, *Methanococcoides methylutens*, *Methanohalobium evestigatum*, *Methanolobus tindarius*, *Methanosarcina sicilia*, *Methanothermus fervidus*, *Petrotoga mobilis*, *Picrophilus torridus*, *Pseudonocardia thermophila*, *Pyrococcus furiosus*, *Pyrodictium occultum*, *Rhodothermus marinus*, *Slackia heliotrinireducens*, *Staphylothermus marinus*, *Sulfolobus acidocaldarius*, *Thermanaerothrix daxensis*, *Thermoanaerobacter* sp., *Thermoanaerobacter thermohydrosulfuricus*, *Thermoanaerobacter wiegelii*, *Thermoanaerobacterium xylanolyticum*, *Thermobifida halotolerans*, *Thermococcus celer*, *Thermococcus litoralis*, *Thermococcus profundus*, *Thermocrinis minervae*, *Thermocrinis ruber*, *Thermoflexus hugenholzii*, *Thermotoga lettingae*, *Thermotoga neapolitana*, *Thermotoga petrophilia*, *Thermus amyloliquefaciens*, *Thermus filiformis*, *Thermus thermophilus*, or *Truepera radiovictrix*, but is not limited thereto.

As used herein, the “psicose-6-phosphate” is known as allulose-6-phosphate, and the “psicose-6-phosphate phosphatase” is an enzyme that catalyzes the production of psicose by dephosphorylating the phosphate group of psicose-6-phosphate.

The psicose-6-phosphate phosphatase of the present application can be used to produce psicose with high efficiency in combination with starch-processing enzymes and sugar phosphate-converting enzymes, in the preparation of psicose by decomposing high-concentration starch.

The psicose-6-phosphate phosphatase of the present application may have an amino acid sequence of any one of SEQ ID NOS: 1 to 222, or may include an amino acid sequence having a homology of at least 70% to the amino acid sequence.

Additionally, it may have an amino acid sequence of any one of SEQ ID NOS: 1, 6, 9,

12, 26, 29, 38 to 43, 45 to 53, 56, 57, 59, 60, 64 to 66, 69, 70, 72, 76, 80, 81, 91 to 93, 95, 99 to 103, 113, 114, 116, 117, 131, 134, 136, 142, 145, 146, 148, 164, 167, 169, 172, 177, 184 to 187, 189, 191, 192, 211, 217, and 221, or may include an amino acid sequence having a homology of at least 70% to the amino acid sequence.

More specifically, it may have an amino acid sequence of any one of SEQ ID NOS: 26, 29, 53, 56, 60, 70, 76, 80, 81, 116, 117, 131, 134, 145, 167, 185, 186, and 191, or may include an amino acid sequence having a homology of at least 70% to the amino acid sequence, but is not limited thereto.

Further, any sequences having the same activity as the amino acid sequence may be included without limitation. In addition, it may include an amino acid sequence of any one of SEQ ID NOS: 1 to 222 or an amino acid sequence having at least 80% homology or identity thereto, but is not limited thereto. Specifically, the amino acid may include an amino acid having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% homology or identity thereto. Furthermore, it is apparent that any protein having an amino acid sequence, in which part of the amino acid sequence is deleted, modified, substituted, or added, may also fall within the scope of the present application as long as it includes an amino acid sequence having such a homology or identity and exhibiting an effect corresponding to that of the above protein.

That is, in the present application, although it is described as “a protein having an amino acid sequence of a particular SEQ ID NO”, it is apparent that any protein, in which part of the amino acid sequence is deleted, modified, substituted, conservatively substituted, or added, may also be used in the present application as long as the protein has the same or corresponding activity to the protein composed of the amino acid sequence of the corresponding SEQ ID NO. For example, as long as a protein has activity identical or corresponding to that of the enzyme, addition of a sequence that does not alter the function of the protein upstream and downstream of the amino acid sequence, naturally occurring mutations, silent mutations, or conservative substitutions thereof are not excluded. It is apparent that even though the protein has such a sequence addition or mutation, it falls within the scope of the present application.

As used herein, the term “homology” or “identity” refers to a degree of relatedness between two given amino acid sequences or nucleotide sequences, and may be expressed as a percentage.

The terms homology and identity may often be used interchangeably with each other.

The sequence homology or identity of the conserved polynucleotide or polypeptide may be determined using standard alignment algorithms, and may be used with default gap penalties established by the program being used. Substantially, homologous or identical sequences may hybridize under moderately or highly stringent conditions such that the full length of the sequence or at least about 50%, 60%, 70%, 80%, or 90% or more of the full length may hybridize. Polynucleotides that contain degenerate codons instead of codons in hybridizing polynucleotides are also considered.

Whether any two polynucleotide sequences have homology, similarity, or identity may be, for example, determined by a known computer algorithm such as the “FASTA” program (Pearson *et al.*, (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444) using default parameters. Alternatively, it may be determined by the Needleman–Wunsch algorithm (Needleman and Wunsch, 1970, *J. Mol. Biol.* 48: 443–453), which is performed using the Needleman program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, *Trends Genet.* 16:276–277) (preferably, version 5.0.0 or versions thereafter) (GCG program package (Devereux, J. *et al.*, *Nucleic Acids Research* 12:387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S. F. *et al.*, *J MOLEC BIOL* 215:403 (1990); *Guide to Huge Computers*, Martin J. Bishop, ed., Academic Press, San Diego, 1994; and CARILLO *et al.* (1988) *SIAM J Applied Math* 48:1073). For example, the homology, similarity, or identity may be determined using BLAST or ClustalW of the National Center for Biotechnology Information (NCBI).

The homology, similarity, or identity of polynucleotides or polypeptides may be determined by comparing sequence information using, for example, the GAP computer program, such as Needleman *et al.* (1970), *J Mol Biol.* 48:443 as disclosed in Smith and Waterman, *Adv. Appl. Math* (1981) 2:482. In summary, the GAP program defines the homology, similarity, or identity as the value obtained by dividing the number of similarly aligned symbols (*i.e.*, nucleotides or amino acids) by the total number of the symbols in the shorter of the two sequences. Default parameters for the GAP program may include (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted comparison matrix of Gribskov *et al.* (1986), *Nucl. Acids Res.* 14:6745, as disclosed in Schwartz and Dayhoff, eds., *Atlas of Protein Sequence and Structure*, National Biomedical Research Foundation, pp. 353–358 (1979) (or EDNAFULL substitution matrix (EMBOSS version of NCBI NUC4.4)); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap (or a gap opening penalty of 10 and a gap extension penalty of 0.5); and (3) no penalty for end gaps. Therefore, as used herein, the term “homology” or “identity”

represents relatedness between sequences.

The psicose-6-phosphate phosphatase of the present application may be an enzyme that selectively catalyzes the dephosphorylation of psicose-6-phosphate. Specifically, the psicose-6-phosphate phosphatase may be an enzyme that catalyzes the dephosphorylation of psicose-6-phosphate when it is mixed with glucose-1-phosphate, glucose-6-phosphate, or fructose-6-phosphate. For example, when the psicose-6-phosphate phosphatase is mixed with the same amount of psicose-6-phosphate, glucose-1-phosphate, glucose-6-phosphate, and fructose-6-phosphate, the dephosphorylation rate of psicose-6-phosphate is 1% or more, 10% or more, or 30% or more. Due to the selective activity of the psicose-6-phosphate phosphatase of the present application, a high rate of psicose conversion can be exhibited in one-pot enzymatic conversion using a plurality of enzymes and substrates at the same time.

The psicose-6-phosphate phosphatase of the present application may be produced by transforming a strain with the enzyme itself or DNA expressing the enzyme, culturing the transformed strain to obtain a culture, and disrupting the culture, followed by purification via column chromatography. The strain for transformation may be *Escherichia coli*, *Corynebacterium glutamicum*, *Aspergillus oryzae*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Pichia pastoris*, or *Bacillus subtilis*, but is not limited thereto, and it may have potential to be transformed into GRAS (Generally Recognized as Safe) strains thereafter.

Another aspect of the present application provides a nucleic acid encoding the psicose-6-phosphate phosphatase, or a vector including the nucleic acid.

As used herein, the term “nucleic acid” has a meaning which collectively includes DNA or RNA molecules. Nucleotides, which are the basic structural units of the nucleic acids, include not only natural nucleotides but also modified analogs thereof in which sugar or base sites are modified (see Scheit, *Nucleotide Analogs*, John Wiley, New York (1980); Uhlman and Peyman, *Chemical Reviews*, 90:543–584 (1990)).

The nucleic acid encoding the enzyme of the present application may be a DNA or RNA sequence in which nucleotides as a unit are covalently linked, and specifically, it may be any one nucleotide sequence among all possible numbers at the time of DNA conversion of the amino acid sequence of SEQ ID NOS: 1 to 222 (modification of amino acids into 61 codons), and more specifically it may include a nucleic acid capable of being translated to exhibit a desired enzymatic activity while having at least 90% or more, 95% or more, 97% or more, 99% or more,

or 100% homology, similarity, or identity to each nucleotide that can be translated into any one of the amino acid sequences of SEQ ID NOS: 1 to 222 of the present application. It is apparent that proteins having the same activity due to codon degeneracy, proteins having the same amino acid sequences after being transcribed, specifically, proteins consisting of any one of the amino acid sequences of SEQ ID NOS: 1 to 222, or polynucleotides that can be translated into proteins having a homology, similarity, or identity thereto are also within the scope of the present application. More specifically, the nucleic acid sequence of the present application is not separately shown, and may be composed of all number of DNA codon that can be translated into the amino acid sequences of SEQ ID NOS: 1 to 222, but is not limited thereto.

Additionally, a probe that may be prepared from a known gene sequence, for example, any sequence which can hybridize with a sequence complementary to all or part of the nucleotide sequence under stringent conditions to encode the enzyme of the present application, may be included without limitation.

The “stringent conditions” refer to conditions under which specific hybridization between polynucleotides is allowed. Such conditions are specifically described in the literature (see J. Sambrook *et al.*, *supra* 9.50–9.51, 11.7–11.8). For example, the stringent conditions may include conditions under which genes having a high homology or identity of 80% or higher, 85% or higher, specifically 90% or higher, more specifically 95% or higher, much more specifically 97% or higher, still much more specifically 99% or higher are hybridized with each other, and genes having a homology or identity lower than the above homologies or identities are not hybridized with each other, or washing conditions of Southern hybridization, that is, washing once, specifically twice or three times at a salt concentration and a temperature corresponding to 60°C, 1× SSC, 0.1% SDS, specifically 60°C, 0.1× SSC, 0.1% SDS, and more specifically 68°C, 0.1× SSC, 0.1% SDS.

Hybridization requires that two nucleic acids contain complementary sequences, although mismatches between bases are possible depending on the stringency of the hybridization. The term “complementary” is used to describe the relationship between nucleotide bases that can hybridize with each other. For example, with respect to DNA, adenine is complementary to thymine, and cytosine is complementary to guanine. Therefore, the present application may include isolated nucleotide fragments complementary to the entire sequence as well as nucleic acid sequences substantially similar thereto.

Specifically, the polynucleotides having a homology or identity may be detected using the hybridization conditions including a hybridization step at a T_m value of 55°C under the

above-described conditions. Further, the T_m value may be 60°C, 63°C, or 65°C, but is not limited thereto, and may be appropriately adjusted by those skilled in the art depending on the purpose thereof.

The appropriate stringency for hybridizing polynucleotides depends on the length of the polynucleotides and the degree of complementation, and these variables are well known in the art (see Sambrook *et al.*, *supra*, 9.50–9.51, 11.7–11.8).

As used herein, the term “vector” may refer to a DNA construct containing the nucleotide sequence of a nucleic acid encoding the enzyme of the present application, which is operably linked to a suitable regulatory sequence such that the target modified protein can be expressed in an appropriate host. The regulatory sequence may include a promoter capable of initiating transcription, any operator sequence for regulating the transcription, a sequence encoding an appropriate mRNA ribosome binding domain, and a sequence regulating the termination of transcription and translation. After being transformed into a suitable host cell, the vector may be replicated or function irrespective of the host genome, and may be integrated into the host genome itself.

The vector used in the present application is not particularly limited as long as it is able to replicate in the host cell, and any vector known in the art may be used. Examples of the vector conventionally used may include natural or recombinant plasmids, cosmids, viruses, and bacteriophages. For example, as a phage vector or cosmid vector, pWE15, M13, MBL3, MBL4, IXII, ASHII, APII, t10, t11, Charon4A, Charon21A, *etc.* may be used; and as a plasmid vector, those based on pBR, pUC, pBluescriptII, pGEM, pTZ, pCL, pET, *etc.* and may be used. Specifically, pDZ, pACYC177, pACYC184, pCL, pECCG117, pUC19, pBR322, pMW118, and pCC1BAC vectors may be used.

Still another aspect of the present application provides a transformant including a vector including a nucleic acid encoding the enzyme of the present application.

As used herein, the “transformant including the nucleic acid encoding the enzyme” or “transformant including the vector including the nucleic acid encoding the enzyme” may refer to a microorganism that has been recombined so that the psicose-6-phosphate phosphatase of the present application is expressed. For example, it may refer to a host cell or a microorganism containing a nucleic acid encoding the psicose-6-phosphate phosphatase, or which is transformed with a vector containing a nucleic acid encoding psicose-6-phosphate phosphatase so that the psicose-6-phosphate phosphatase is expressed. For the purpose of the present application, the psicose-6-phosphate phosphatase expressed by the transformant may be composed of any one

amino acid sequence of SEQ ID NOS: 1 to 222, but is not limited thereto.

As used herein, the term “transformation” refers to the introduction of a vector including a nucleic acid encoding the psicose-6-phosphate phosphatase into a host cell so that the protein encoded by the nucleic acid can be expressed in the host cell. As long as the transformed nucleic acid can be expressed in a host cell, it does not matter whether it is integrated into the chromosome of the host cell and located therein or located extrachromosomally, and both cases can be included. Further, the nucleic acid may include DNA and RNA encoding the nucleic acid encoding the psicose-6-phosphate phosphatase of the present application. The nucleic acid may be introduced in any form, as long as it can be introduced into the host cell and expressed therein. For example, the nucleic acid may be introduced into the host cell in the form of an expression cassette, which is a gene construct including all elements required for its autonomous expression. The expression cassette may commonly include a promoter operably linked to the nucleic acid, a transcription terminator, a ribosome binding domain, or a translation terminator. The expression cassette may be in the form of a self-replicable expression vector. Additionally, the nucleic acid may be introduced into the host cell as it is and operably linked to sequences required for expression in the host cell, but is not limited thereto.

In addition, as used herein, the term “operably linked” means that the gene sequence is functionally linked to a promoter sequence that initiates and mediates transcription of the nucleic acid encoding the psicose-6-phosphate phosphatase of the present application.

The insertion of the nucleic acid or the vector into the chromosome may be performed by any method known in the art, for example, by homologous recombination, but the method is not limited thereto. Additionally, the vector may further include a selection marker to confirm the insertion into the chromosome. The selection marker is for selecting the cells transformed with the vector, that is, for confirming whether the target nucleic acid molecule has been inserted, and markers that provide selectable phenotypes, such as drug resistance, auxotrophy, resistance to cell-toxic agents, or expression of surface modified proteins, may be used. When treated with a selective agent, only the cells expressing the selection marker can survive or express other phenotypic traits, and thus the transformed cells can be selected.

The method of transforming the vector of the present application includes any method of introducing a nucleic acid into a cell, and may be performed by selecting a suitable standard technique known in the art, depending on the host cell. For example, the method may include electroporation, calcium phosphate (CaPO_4) precipitation, calcium chloride (CaCl_2) precipitation, retroviral infection, microinjection, a polyethyleneglycol (PEG) method, a DEAE-dextran

method, a cationic liposome method, and a lithium acetate–DMSO method, but is not limited thereto.

As the host cell, it is preferable to use a host having a high efficiency of introducing DNA and a high efficiency of expressing the introduced DNA. For example, it may be a microorganism of the genus *Corynebacterium*, a microorganism of the genus *Escherichia*, a microorganism of the genus *Serratia*, a microorganism of the genus *Bacillus*, a microorganism of the genus *Saccharomyces*, or a microorganism of the genus *Pichia*, and specifically, it may be *E. coli*, but is not limited thereto, and may be applied to all GRAS strains.

More specifically, the transformant of the present application may be 222 in total, from *E. coli* BL21(DE3)/pET-CJ-ap1 to *E. coli* BL21(DE3)/pET-CJ-ap222.

Yet another aspect of the present application provides a composition for producing psicose including the psicose-6-phosphate phosphatase, a microorganism expressing the same, or a culture of the microorganism. Specifically, the composition may further include a sugar phosphate, and more specifically, it may further include psicose-6-phosphate as a substrate, but is not limited thereto.

The composition for producing psicose of the present application may produce psicose through the dephosphorylation of psicose-6-phosphate by including the psicose-6-phosphate phosphatase which acts to produce psicose by dephosphorylating psicose-6-phosphate, a microorganism expressing the same, or a culture of the microorganism.

Additionally, the composition for producing psicose of the present application may further include an enzyme and/or a substrate involved in the psicose production pathway of the present application [(i) starch, maltodextrin, sucrose, or a combination thereof; (ii) a phosphate or polyphosphate or other phosphorylating compounds; (iii) a fructose-6-phosphate-3-epimerase; (iv) a glucose-6-phosphate-isomerase; (v) a phosphoglucomutase or a glucokinase; and/or (vi) an α -glucan phosphorylase, a starch phosphorylase, a maltodextrin phosphorylase, a sucrose phosphorylase, an α -amylase, a pullulanase, an isoamylase, a glucoamylase, an α -glucanotransferase, a polyphosphate glucokinase, or a sucrase]; a microorganism expressing the enzymes involved in the psicose production pathway; or a culture of the microorganism expressing the enzymes involved in the psicose production pathway. However, the enzymes that are included in the composition for producing psicose of the present application and the substrates used in the production of psicose are merely illustrative and are not limited as long as psicose can be produced using the psicose-6-phosphate phosphatase of the present application.

Specifically, the fructose-6-phosphate-3-epimerase may include any protein that is active in converting fructose-6-phosphate to psicose-6-phosphate. The glucose-6-phosphate-isomerase may include any protein that is active in converting glucose-6-phosphate to fructose-6-phosphate. The phosphoglucomutase (EC 5.4.2.2) may include any protein that is active in converting glucose-1-phosphate to glucose-6-phosphate. The starch/maltodextrin phosphorylase (EC 2.4.1.1) and α -glucan phosphorylase may include any protein that is active in phosphoryl transfer to glucose to produce glucose-1-phosphate from starch or maltodextrin. The sucrose phosphorylase (EC 2.4.1.7) may include any protein that is active in phosphoryl transfer to glucose to produce glucose-1-phosphate from sucrose. The α -amylase (EC 3.2.1.1), pullulanase (EC 3.2.1.41), isoamylase (EC 3.2.1.68), 4- α -glucanotransferase (EC 2.4.1.25), and glucoamylase (EC 3.2.1.3), which are starch-liquefying enzymes, may include any protein that is active in converting starch or maltodextrin to debranched maltooligosaccharide or glucose. The sucrase (EC 3.2.1.26) may include any protein that is active in converting sucrose to glucose. The polyphosphate glucokinase (EC 2.7.1.63) may include any protein that is active in phosphoryl transfer to glucose to convert glucose to glucose-6-phosphate.

The psicose-6-phosphate phosphatase, α -glucan phosphorylase, phosphoglucomutase (or phosphomannomutase), glucose-6-phosphate isomerase, psicose-6-phosphate-3-epimerase (or ribulose-5-phosphate-3-epimerase), pullulanase (or isoamylase), 4- α -glucanotransferase, and polyphosphate glucokinase that are included in the composition for producing psicose of the present application may have little or no side reactions with psicose, which is the final product.

The composition for producing psicose of the present application may properly contain not only the psicose-6-phosphate phosphatase, but also a plurality of enzymes and substrates thereof for preparing psicose. The psicose-6-phosphate phosphatase has the effect of being able to selectively and irreversibly produce psicose from psicose-6-phosphate even in an environment in which several enzymes are present.

The composition for producing psicose of the present application may further include any suitable conventionally used excipients. Examples of such excipients include, for example, preservatives, wetting agents, dispersants, suspending agents, buffers, stabilizers, and isotonic agents, but are not limited thereto.

The composition for producing psicose of the present application may further include a metal ion or a metal salt. In one embodiment, the metal ion may be a divalent metal cation.

Specifically, the metal ion may be at least one selected from the group consisting of Ni, Mg, Co, Mn, Fe, and Zn ions. More specifically, the composition for producing psicose of the present application may further include a metal salt. Even more specifically, the metal salt may be at least one selected from the group consisting of NiSO₄, MgSO₄, MgCl₂, NiCl₂, CoSO₄, CoCl₂, MnCl₂, MnSO₄, FeSO₄, and ZnSO₄.

Even another aspect of the present application provides a method for producing psicose, including converting psicose-6-phosphate into psicose by bringing psicose-6-phosphate into contact with the psicose-6-phosphate phosphatase, a microorganism expressing the same, or a culture of the microorganism.

Specifically, in the method for producing psicose, psicose may be produced by bringing psicose-6-phosphate into contact with the psicose-6-phosphate phosphatase, a microorganism expressing the same, or a culture of the microorganism, but is not limited thereto.

The method of the present application may further include converting fructose-6-phosphate into psicose-6-phosphate by bringing fructose-6-phosphate into contact with fructose-6-phosphate-3-epimerase, a microorganism expressing the fructose-6-phosphate-3-epimerase, or a culture of the microorganism expressing the fructose-6-phosphate-3-epimerase, prior to the step of converting psicose-6-phosphate to psicose.

The method of the present application may further include converting glucose-6-phosphate into fructose-6-phosphate by bringing glucose-6-phosphate into contact with glucose-6-phosphate-isomerase, a microorganism expressing the glucose-6-phosphate-isomerase, or a culture of the microorganism expressing the glucose-6-phosphate-isomerase, prior to the step of converting fructose-6-phosphate to psicose-6-phosphate.

The method of the present application may further include converting glucose-1-phosphate into glucose-6-phosphate by bringing glucose-1-phosphate into contact with phosphoglucomutase, a microorganism expressing the phosphoglucomutase, or a culture of the microorganism expressing the phosphoglucomutase, prior to the step of converting glucose-6-phosphate into fructose-6-phosphate.

The method of the present application may further include converting glucose into glucose-6-phosphate by bringing glucose into contact with polyphosphate glucokinase, a microorganism expressing the polyphosphate glucokinase, or a culture of the microorganism expressing the polyphosphate glucokinase, and polyphosphate, prior to the step of converting

glucose-6-phosphate into fructose-6-phosphate.

The method of the present application may further include converting starch, maltodextrin, sucrose, or a combination thereof into glucose-1-phosphate by bringing starch, maltodextrin, sucrose, or a combination thereof into contact with α -glucan phosphorylase, starch phosphorylase, maltodextrin phosphorylase, or sucrose phosphorylase; a microorganism expressing the phosphorylase; or a culture of the microorganism expressing the phosphorylase, and phosphate, prior to the step of converting glucose-1-phosphate into glucose-6-phosphate.

The method of the present application may further include converting starch, maltodextrin, sucrose, or a combination thereof into glucose by bringing starch, maltodextrin, sucrose, or a combination thereof into contact with α -amylase, pullulanase, isoamylase, glucoamylase, or sucrase; a microorganism expressing the α -amylase, pullulanase, glucoamylase, sucrase, or isoamylase; or a culture of the microorganism expressing the α -amylase, pullulanase, glucoamylase, sucrase, or isoamylase, prior to converting starch, maltodextrin, sucrose, or a combination thereof into glucose-1-phosphate.

The psicose-6-phosphate phosphatase, α -glucan phosphorylase, phosphoglucomutase (or phosphomannomutase), glucose-6-phosphate isomerase, psicose-6-phosphate-3-epimerase (or ribulose-5-phosphate-3-epimerase), pullulanase (or isoamylase), 4- α -glucanotransferase, and polyphosphate glucokinase that are used in the method for producing psicose of the present application may have little or no side reactions with psicose, which is the final product.

In the method for producing psicose of the present application, the optimal/maximum psicose may be produced in a complex combination with sugar phosphate-converting enzymes by decomposing a high concentration of starch, and a maximum of 8 kinds of enzymes may be used in combination to secure the maximum productivity of psicose.

First, glucan phosphorylase (glycogen phosphorylase, EC 2.4.1.1), an enzyme that degrades starch and produces glucose-1-phosphate, specifically produces glucose-1-phosphate on α -1,4-bound starch. Secondly, phosphoglucomutase (EC 2.7.5.1) or phosphomannomutase (EC 5.4.2.8), which converts the thus-produced glucose-1-phosphate into glucose-6-phosphate, is used in the intermediate complex enzyme reaction. As the third enzyme used, glucose-6-phosphate isomerase (EC 5.3.1.9), which converts glucose-6-phosphate to fructose-6-phosphate, is used. Fourth, ribulose-5-phosphate-3-epimerase or psicose-6-phosphate-3-epimerase, which is the enzyme that converts fructose-6-phosphate to psicose-6-phosphate as described above, is used to produce psicose-6-phosphate in a reversible reaction. In the reaction of starch to psicose-6-phosphate, although it is impossible to produce more than a certain amount in the reversible reaction, the use of the selective

psicose-6-phosphate phosphatase included in the present invention enables the production of psicose in high yield.

In order to further increase the starch utilization rate, pullulanase (EC 3.2.1.41) or isoamylase (EC 3.2.1.68) is used together to degrade branched α -1,6 bonds in addition to the α -1,4 bonds of amylopectin. Additionally, in order to increase the starch utilization of glucan phosphorylase, 4- α -glucanotransferase (EC 2.4.1.25) is used. The utilization of the segmented starch substrate can be increased by binding oligosaccharides in the form of α -1,4 bonds to maltose or other oligosaccharides, which are substrates with relatively low activity. In addition, polyphosphate-glucose phosphotransferase (EC 2.7.1.63) can be used to produce additional psicose through complex enzymatic reactions from glucose degraded after the use of starch, thereby securing the maximum psicose conversion rate.

Further, in the method of the present application, the contact may be carried out at a pH of 5.0 to 9.0, specifically at a pH of 6.0 to 8.0.

In the method of the present application, the contact may be carried out at a temperature of 40°C to 80°C, specifically at a temperature of 40°C to 60°C or 50°C to 60°C.

In the method of the present application, the contact may be carried out for 2 hours to 24 hours, specifically for 6 to 24 hours or 120 hours.

In the method of the present application, the contact may be carried out at a pH of 5.0 to 9.0, at a temperature of 40°C to 80°C, and/or for 2 hours to 24 hours. Specifically, the contact may be carried out at a pH of 6.0 to 8.0, at a temperature of 40°C to 60°C or 50°C to 60°C, and/or for 6 hours to 24 hours or 120 hours.

The method of the present application may further include purifying psicose. There is no particular limitation on the method for purifying psicose, and any suitable method known in the art may be used. Non-limiting examples of such purification methods include chromatography, fractional crystallization, and ion purification, which may be carried out alone or in a combination of two or more thereof. For example, the reaction product of psicose may be purified by chromatography. In this case, the purification of saccharides by chromatography may be performed based on small differences in binding force between saccharides to be purified and metal ions attached to an ionic resin.

The method of the present application may further include bleaching, demineralizing, or both before or after the purification step. The bleaching and/or demineralizing enables production of purer psicose without impurities.

[Mode for Carrying Out the Invention]

Hereinafter, the present application will be described in more detail by way of Examples. However, these Examples are provided for illustrative purposes only to aid in the understanding of the present application, and the scope of the present application is not intended to be limited to or by these Examples.

In the present application, amino acids may be represented by the following abbreviations or amino acid names.

[Table 1]

Type of amino acids	Abbrevi- -ation	DNA codon encoded by corresponding amino acid	RNA codon encoded by corresponding amino acid
alanine	A	GCT, GCC, GCA, GCG	GCU, GCC, GCA, GCG
arginine	R	AGA, AGG	AGA, AGG
asparagine	N	AAT, AAC	AAU, AAC
aspartic acid	D	GAT, GAC	GAU, GAC
cystein	C	TGT, TGC	UGU, UGC
glutamic acid	E	GAA, GAG	GAA, GAG
glutamine	Q	CAA, CAG	CAA, CAG
glycine	G	GGT, GGC, GGA, GGG	GGU, GGC, GGA, GGG
histidine	H	CAC, CAT	CAC, CAU
isoleucine	I	ATT, ATC, ATA	AUU, AUC, AUA
leucine	L	TTA, TTG, CTT, CTC, CTA, CTG	UUA, UUG, CUU, CUC, CUA, CUG
lysine	K	AAA, AAG	AAA, AAG
methionine	M	ATG	AUG
phenylalanine	F	TTT, TTC	UUU, UUC
proline	P	CCT, CCC, CCA, CCG	CCU, CCC, CCA, CCG
serine	S	TCT, TCC, TCA, TCG	UCU, UCC, UCA, UCG
threonine	T	ACT, ACC, ACA, ACG	ACU, ACC, ACA, ACG
tryptophan	W	TGG	UGG
tyrosine	Y	TAT, TAC	UAU, UAC
valine	V	GTT, GTC, GTA, GTG	GUU, GUC, GUA, GUG

Example 1: Preparation of Recombinant Expression Vector of Each Enzyme and Transformed Microorganism

In order to provide the enzymes necessary for the psicose production pathway, thermoresistant genes were selected, which are summarized in Table 2 below.

[Table 2]

SEQ ID NO	Species and Genus	NCBI No.
1	<i>Alicyclobacillus acidocaldarius</i>	ACV59297.1
2	<i>Alicyclobacillus tengchongensis</i>	KRW92667.1
3		WP_058093972.1
4	<i>Amycolatopsis thermoflava</i>	WP_017986193.1
5		WP_037323890.1
6	<i>Anaerolinea thermolimosa</i>	WP_084001059.1
7		WP_062189018.1
8		GAP08215.1
9	<i>Anaerolinea thermophila</i>	BAJ62794.1
10		KUX46216.1
11		BAJ63987.1
12	<i>Archaeoglobus fulgidus</i>	AAB91288.1
13		AAB89113.1
14		AAB89416.1
15	<i>Archaeoglobus profundus</i>	WP_012940213.1
16	<i>Archaeoglobus veneficus</i>	WP_013683862.1
17	<i>Bacillus licheniformis</i>	AAU41557.1
18		AAU39603.1
19		AAU43073.1
20	<i>Caldicellulosiruptor bescii</i>	ACM59531.1
21		ACM59457.1
22		ACM61198.1
23		ACM61211.1
24		ACM60182.1
25	<i>Caldilinea aerophila</i>	BAL98676.1
26		BAM00874.1
27		WP_014433277.1
28	<i>Caldithrix abyssi</i>	APF18124.1
29		APF18744.1
30		APF16831.1
31		WP_006929206.1

32	<i>Carboxydocella</i> sp. UL01	WP_079933669.1
33	<i>Carboxydotherrnus ferrireducens</i>	WP_028051691.1
34		WP_028051711.1
35		WP_028053062.1
36	<i>Chloroflexi bacterium</i> 54-19	OJW02856.1
37	<i>Defluviitoga tunisiensis</i>	WP_045087739.1
38	<i>Deinococcus</i> <i>serius</i>	WP_103127908.1
39	<i>Deinococcus</i> <i>apschensis</i>	WP_019584763.1
40	<i>Deinococcus</i> <i>aquatilis</i>	WP_019011981.1
41	<i>Deinococcus</i> <i>geothermalis</i>	WP_011529832.1
42	<i>Deinococcus</i> <i>hopiensis</i>	WP_084049191.1
43	<i>Deinococcus</i> <i>maricopensis</i>	WP_013556873.1
44	<i>Deinococcus</i> <i>murrayi</i>	WP_084542862.1
45		WP_051363537.1
46	<i>Deinococcus</i> <i>reticulitermitis</i>	WP_092263046.1
47	<i>Deinococcus</i> <i>wulumuqiensis</i>	WP_017870657.1
48	<i>Deinococcus</i> sp. Leaf326	WP_056297006.1
49	<i>Deinococcus</i> <i>phoenicis</i>	WP_034353069.1
50	<i>Deinococcus</i> <i>proteolyticus</i>	WP_013614672.1
51	<i>Deinococcus</i> sp. 17bor-2	WP_109826642.1
52	<i>Deinococcus</i> sp. NW-56	WP_104990527.1
53	<i>Deinococcus</i> sp. RL	KEF35131.1
54		WP_081851636.1
55	<i>Deinococcus</i> sp. YIM 77859	WP_034384462.1
56	<i>Desulfurococcus</i> <i>mucosus</i>	ADV64381.1
57		ADV64382.1
58		ADV64566.1
59	<i>Dictyoglomus</i> <i>turgidum</i>	YP_002352793.1
60		YP_002353123.1
61		YP_002352154.1
62	<i>Effusibacillus</i> <i>pohlise</i>	WP_018132070.1
63	<i>Fervidobacterium</i> <i>gondwanense</i>	WP_072759218.1
64	<i>Fervidobacterium</i> <i>islandicum</i>	AMW32328.1
65		AMW33413.1

66	<i>Fervidobacterium nodosum</i>	ABS60322.1
67		ABS61294.1
68	<i>Fervidobacterium pennivorans</i>	AFG34634.1
69		AFG35917.1
70		WP_064011782.1
71	<i>Geobacillus sp.</i>	WP_011232549.1
72	<i>Geobacillus stearothermophilus</i>	ALA69320.1
73		ALA71677.1
74		ALA71036.1
75		ALA70927.1
76		ALA69332.1
77	<i>Halococcus salifodinae</i>	WP_005045395.1
78		EMA49396.1
79		EMA52756.1
80	<i>Hydrogenivirga sp. 128-5-B1-1</i>	WP_008285887.1
81	<i>Hydrogenobacter hydrogenophilus</i>	WP_096602199.1
82		WP_096601840.1
83	<i>Hydrogenobacter thermophilus</i>	WP_012963417.1
84	<i>Hyperthermus butylicus</i>	ABM80367.1
85	<i>Kosmotoga arenicorallina</i>	WP_084251417.1
86	<i>Kosmotoga olearia</i>	ACB80305.1
87		ACB80819.1
88	<i>Narinitoga piezophila</i>	AEX84499.1
89		AEX85453.1
90		AEX85799.1
91	<i>Neiothermus cerbereus</i>	WP_027877060.1
92		WP_027878515.1
93	<i>Neiothermus chliarophilus</i>	WP_027892542.1
94		WP_051304156.1
95	<i>Neiothermus ruber</i>	ADD26908.1
96		WP_013013826.1
97		WP_013013825.1
98		WP_013012548.1
99		WP_027883570.1
100	<i>Neiothermus silvanus</i>	ADH63424.1
101		WP_013157023.1
102	<i>Neiothermus taiwanensis</i>	WP_027888084.1
103		WP_027888305.1

104	<i>Neiothermus timidus</i>	WP_018466213.1
105		WP_018465080.1
106		WP_018467688.1
107		WP_018467518.1
108		WP_018465603.1
109		WP_018466116.1
110		WP_018467797.1
111		WP_018467420.1
112		WP_018466214.1
113		WP_018465420.1
114	<i>Neiothermus rufus</i>	WP_036271046.1
115	<i>Nesotoga infera</i>	EUK90417.1
116	<i>Metallospira sedula</i>	ABP94905.1
117	<i>Methanocella conradii</i>	AFC99407.1
118		WP_014405429.1
119		AFD00405.1
120	<i>Methanococcoides methylutens</i>	WP_048194361.1
121		AKB84306.1
122	<i>Methanohalobium evestigatum</i>	ADI75005.1
123		WP_013193898.1
124	<i>Methanlobus tindarius</i>	WP_048135864.1
125		WP_048135649.1
126		ETA67773.1
127	<i>Methanosarcina siciliae</i>	WP_048179383.1
128		AKB37722.1
129		AKB35909.1
130	<i>Methanothermobacter formicivorans</i>	ADP76907.1
131	<i>Petrogaster mobilis</i>	AKK30802.1
132		EUK15285.1
133	<i>Picrophilus torridus</i>	AA744010.1
134		AA743693.1
135	<i>Pseudocardia thermophila</i>	WP_073456926.1

136	<i>Pyrococcus furiosus</i>	AAI80556.1
137		AAI81109.1
138		WP_014835302.1
139		AAI82045.1
140		AAI82122.1
141		AAI81285.1
142		AAI81790.1
143		AAI80346.1
144		AAI80703.1
145		AAI81901.1
146		AAI81913.1
147		AAI80587.1
148		AFN04628.1
149	<i>Pyrodicticum occultum</i>	WP_083494490.1
150	<i>Rhodothermus marinus</i>	WP_041806336.1
151		WP_012844425.1
152		ACY47561.1
153		ACY48134.1
154		ACY47672.1
155		ACY48911.1
156		WP_012843092.1
157		WP_012843709.1
158		WP_012844300.1
159		ACY48106.1
160		WP_012844667.1
161		WP_012845116.1
162		WP_012844208.1
163		WP_012843855.1
164		ACY47289.1
165	<i>Slackia heliotrinireducens</i>	WP_012798605.1
166		ACY22623.1
167	<i>Staphylothermus marinus</i>	ABN70247.1
168		ABN69637.1
169		ABN69899.1
170	<i>Sulfolobus acidocaldarius</i>	AAI79446.1
171		WP_015385352.1
172		AAI80292.1

173	<i>Thermaerothrix daxensis</i>	EPL83857.1
174	<i>Thermoanaerobacter</i> sp.	WP_004400108.1
179	<i>Thermoanaerobacterium xylanolyticum</i>	WP_013788008.1
180	<i>Thermobifida halotolerans</i>	WP_068689397.1
181		WP_068692137.1
182	<i>Thermococcus celer</i>	WP_088862065.1
183		ASI98087.1
184	<i>Thermococcus litoralis</i>	EHR78031.1
185	<i>Thermococcus profundus</i>	WP_088857759.1
186	<i>Thermocrinis minervae</i>	WP_079653666.1
187	<i>Thermocrinis ruber</i>	AHE95294.1
188	<i>Thermoflexus hugenholtzii</i>	WP_088571736.1
189	<i>Thermotoga lettingae</i>	ABV34293.1
190	<i>Thermotoga neapolitana</i>	WP_015919708.1
191		ACM22182.1
192		ACM22192.1
193		ACM22252.1
194		ACM22284.1
195		ACM22382.1
196		ACM22666.1
197		ACM22704.1
198		ACM22708.1
199		ACM22832.1
200		ACM22990.1
201		ACM23016.1
202		ACM23040.1
203		ACM23081.1
204		ACM23138.1
205		ACM23254.1
206		ACM23323.1
207		ACM23341.1
208		ACM23415.1
209		ACM23510.1
210		ACM23571.1
211		ACM23575.1
212		ACM23847.1
213		ACM23859.1
214		ACM23950.1
215		ACM23957.1
216		ACM24018.1

217	<i>Thermotoga petrophilia</i>	ABQ46309.1
218	<i>Thermus amyloliquefaciens</i>	WP_038055695.1
219	<i>Thermus filiformis</i>	WP_038062097.1
220	<i>Thermus thermophilus</i>	WP_096411270.1
221		AAS82270.1
222	<i>Truepera radiovictrix</i>	WP_013176662.1

The genes of the selected amino acids were amplified by way of polymerase chain reaction (PCR) using gene synthesis or chromosomal DNA (genomic DNA) of each cultured strain, and the amplified DNA was inserted into plasmid vector pET2la (Novagen) for *E. coli* expression using restriction enzymes NdeI and XhoI or SalI to construct recombinant expression vectors. The expression vectors were transformed into strain *E. coli* BL21(DE3) using a general transformation technique (see Sambrook *et al.* 1989) to produce transformed microorganisms.

Specifically, the psicose-6-phosphate phosphatase of SEQ ID NOS: 26, 29, 53, 56, 60, 70, 76, 80, 81, 116, 117, 131, 134, 145, 167, 185, 186, and 191 was transformed into the *E. coli* BL21(DE3) strain to prepare transformed microorganisms, which were named *E. coli* BL21(DE3)/pET-CJ-ap26, *E. coli* BL21(DE3)/pET-CJ-ap29, *E. coli* BL21(DE3)/pET-CJ-ap53, *E. coli* BL21(DE3)/pET-CJ-ap56, *E. coli* BL21(DE3)/pET-CJ-ap60, *E. coli* BL21(DE3)/pET-CJ-ap70, *E. coli* BL21(DE3)/pET-CJ-ap76, *E. coli* BL21(DE3)/pET-CJ-ap80, *E. coli* BL21(DE3)/pET-CJ-ap81, *E. coli* BL21(DE3)/pET-CJ-ap116, *E. coli* BL21(DE3)/pET-CJ-ap117, *E. coli* BL21(DE3)/pET-CJ-ap131, *E. coli* BL21(DE3)/pET-CJ-ap134, *E. coli* BL21(DE3)/pET-CJ-ap145, *E. coli* BL21(DE3)/pET-CJ-ap167, *E. coli* BL21(DE3)/pET-CJ-ap185, *E. coli* BL21(DE3)/pET-CJ-ap186, and *E. coli* BL21(DE3)/pET-CJ-ap191.

The thus-prepared transformed microorganisms were deposited at the Korean Culture Center of Microorganisms (KCCM), an International Depositary Authority, on November 14, 2018 with Accession Nos. KCCM12390P (*E. coli* BL21(DE3)/pET-CJ-ap26), KCCM12391P (*E. coli* BL21(DE3)/pET-CJ-ap29), KCCM12392P (*E. coli* BL21(DE3)/pET-CJ-ap53), KCCM12393P (*E. coli* BL21(DE3)/pET-CJ-ap56), KCCM12394P (*E. coli* BL21(DE3)/pET-CJ-ap60), KCCM12395P (*E. coli* BL21(DE3)/pET-CJ-ap70), KCCM12396P (*E. coli* BL21(DE3)/pET-CJ-ap76), KCCM12397P (*E. coli* BL21(DE3)/pET-CJ-ap80), KCCM12398P (*E. coli* BL21(DE3)/pET-CJ-ap81), KCCM12399P (*E. coli*

BL21(DE3)/pET-CJ-ap116), KCCM12400P (*E. coli* BL21(DE3)/pET-CJ-ap117), KCCM12401P (*E. coli* BL21(DE3)/pET-CJ-ap131), KCCM12402P (*E. coli* BL21(DE3)/pET-CJ-ap134), KCCM12403P (*E. coli* BL21(DE3)/pET-CJ-ap145), KCCM12404P (*E. coli* BL21(DE3)/pET-CJ-ap167), KCCM12405P (*E. coli* BL21(DE3)/pET-CJ-ap185), KCCM12406P (*E. coli* BL21(DE3)/pET-CJ-ap186), and KCCM12407P (*E. coli* BL21(DE3)/pET-CJ-ap191).

Example 2: Preparation of Recombinant Enzymes

In order to prepare recombinant enzymes, a culture tube containing 5 mL of an LB liquid medium was inoculated with each of the transformed microorganisms prepared in Example 1. The seed culture was performed in a shaking incubator at 37°C until an absorbance of 2.0 at 600 nm was reached. The culture broth obtained by way of the seed culture was inoculated to a culture flask containing the LB liquid medium, followed by main culture. When the absorbance of the culture at 600 nm reached 2.0, 1 mM IPTG was added to induce the expression and production of a recombinant enzyme. The culture was performed at a stirring speed of 180 rpm, and the culture temperature was maintained at 37°C. The culture broth was centrifuged at 8,000×*g* and at 4°C for 20 minutes to recover cells. The recovered cells were washed with 50 mM Tris-HCl (pH 8.0) buffer twice and re-suspended in the same buffer, and the cells were disrupted using an ultrasonic homogenizer. The cell lysates were centrifuged at 13,000×*g* and 4°C for 20 minutes to obtain only supernatant. The recombinant enzyme was purified from the supernatant by His-tag affinity chromatography, and the purified recombinant enzyme was dialyzed against 50 mM Tris-HCl buffer (pH 8.0) and was then used for subsequent reaction.

Example 3: Analysis of Dephosphorylation Activity of Sugar Phosphate and Psicose-6-phosphate

After preparing psicose-6-phosphate from fructose-6-phosphate, the activity of psicose production by psicose-6-phosphate phosphatase was confirmed, or the activity was measured by detecting psicose by further adding three enzymes using glucose-1-phosphate as the first substrate. In particular, glucose, fructose, and psicose, which are common sugars produced by mixing each of the 222 phosphatases of the present application with the three enzymes (*i.e.*, phosphoglucomutase (EC 2.7.5.1) or phosphomannomutase (EC 5.4.2.8), which converts glucose-1-phosphate to glucose-6-phosphate; glucose-6-phosphate isomerase (EC 5.3.1.9), which converts glucose-6-phosphate to fructose-6-phosphate; and ribulose-5-phosphate-3-epimerase or psicose-6-phosphate-3-epimerase, which is an enzyme that

converts fructose-6-phosphate to psicose-6-phosphate) were qualitatively and quantitatively evaluated.

Specifically, 50 mM fructose-6-phosphate or 20 mM glucose-1-phosphate was suspended in 50 mM Tris-HCl (pH 7.0), 50 mM sodium phosphate (pH 6 to 7), or 50 mM potassium phosphate (pH 6 to 7), and then 0.1 unit/mL of phosphoglucomutase; phosphomannomutase, glucose-6-phosphate isomerase, and ribulose-5-phosphate-3-epimerase; or psicose-6-phosphate-3-epimerase and each of the 222 recombinant psicose-6-phosphate phosphatases prepared in Example 2 were added thereto, and the mixture was allowed to react at 45°C to 70°C for 1 to 24 hours. The production of glucose, fructose, and psicose was confirmed by HPLC, which was performed using an SP 0810 column (Shodex) and an Aminex HPX-87C column (Bio-RAD) at 80°C with a mobile phase flow rate of 0.6 mL/min, and detected using a refractive index detector (RID).

Thereafter, in the prepared mixed solution of glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, and psicose-6-phosphate, the dephosphorylation activity of the psicose-6-phosphate phosphatase of the present application and the psicose-6-phosphate-specific (selective) dephosphorylation rate were measured.

Specifically, 0.1 unit/mL of each of the psicose-6-phosphate phosphatase and 5 mM MgCl_2 (or MgSO_4) were added to the mixed solution of 1% (w/v) glucose-6-phosphate, glucose-1-phosphate, fructose-6-phosphate, and psicose-6-phosphate, and the mixture was allowed to react at 50°C for 12 hours. The reaction products were analyzed by HPLC using an Aminex HPX-87C column (Bio-RAD), at 80°C with a mobile phase flow rate of 0.6 mL/min. A refractive index detector was used to detect the production of psicose and other saccharides (fructose and glucose).

As a result, among all 222 enzymes, 69 enzymes showed high selective dephosphorylation activity for psicose-6-phosphate, and 45 enzymes showed weak selective dephosphorylation activity for psicose-6-phosphate. Additionally, in 108 enzymes, the dephosphorylation titer for selective psicose-6-phosphate could not be confirmed. However, dephosphorylation activity was confirmed in 221 enzymes excluding one of the 222 enzymes, of which 113 enzymes showed high dephosphorylation activity, and 108 enzymes showed low dephosphorylation activity. Meanwhile, the enzyme of SEQ ID NO: 54, which was confirmed to have no dephosphorylation titer, is known as a dephosphorylation enzyme, but it was confirmed that it has no substantial dephosphorylation activity. The results are summarized in

Table 3 below.

A = Low Activity (Dephosphorylation conversion rate from sugar phosphate to simple sugar is 1–30%)

B = High Activity (Dephosphorylation conversion rate from sugar phosphate to simple sugar is 30% or more)

C = No Activity (Dephosphorylation conversion rate from sugar phosphate to simple sugar is less than 1%)

[Table 3]

SEQ. ID NO	Species and Genus	NCBI No.	G1P/G6P/F6P/ P6P Dephosphorylation activity	P6P(Selective) /G1P+G6P+F6P +P6P Dephosphorylation activity
1	<i>Alicyclobacillus acidocaldarius</i>	ACV59297.1	B	B
2	<i>Alicyclobacillus tengchongensis</i>	KRW92667.1	A	C
3		WP_058093972.1	A	C
4	<i>Amycolatopsis thermoflava</i>	WP_017986193.1	A	C
5		WP_037323890.1	A	C
6	<i>Anaerolinea thermolimosa</i>	WP_084001059.1	B	B
7		WP_062189018.1	A	C
8		GAP08215.1	A	C
9	<i>Anaerolinea thermophila</i>	BAJ62794.1	B	B
10		KUK46216.1	A	C
11		BAJ63987.1	A	C
12	<i>Archaeoglobus fulgidus</i>	AAB91288.1	B	B
13		AAB89113.1	B	A
14		AAB89416.1	B	A
15	<i>Archaeoglobus profundus</i>	WP_012940213.1	A	C
16	<i>Archaeoglobus veneficus</i>	WP_013683862.1	A	C
17	<i>Bacillus licheniformis</i>	AAU41557.1	B	A
18		AAU39603.1	B	A
19		AAU43073.1	A	C
20	<i>Caldicellulosiruptor bescii</i>	ACM59531.1	B	A
21		ACM59457.1	B	A
22		ACM61198.1	B	A
23		ACM61211.1	B	A
24		ACM60182.1	A	C
25	<i>Caldilinea aerophila</i>	BAL98676.1	B	A
26		BAM00874.1	B	B
27		WP_014433277.1	A	C
28	<i>Caldithrix abyssi</i>	APF18124.1	B	A
29		APF18744.1	B	B
30		APF16831.1	A	C
31		WP_006929206.1	A	C
32	<i>Carboxydocella sp. UL01</i>	WP_079933669.1	A	C
33	<i>Carboxydotherrnus ferrireducens</i>	WP_028051691.1	A	C
34		WP_028051711.1	A	C

35		WP_028053062.1	A	C
36	<i>Chloroflexi bacterium</i> 54-19	OJW02856.1	A	C
37	<i>Deffluviitoga tunisiensis</i>	WP_045087739.1	B	A
38	<i>Deinococcus aerius</i>	WP_103127908.1	B	B
39	<i>Deinococcus apachensis</i>	WP_019584763.1	B	B
40	<i>Deinococcus aquatilis</i>	WP_019011981.1	B	B
41	<i>Deinococcus geothermalis</i>	WP_011529832.1	B	B
42	<i>Deinococcus hopiensis</i>	WP_084049191.1	B	B
43	<i>Deinococcus maricopensis</i>	WP_013556873.1	B	B
44	<i>Deinococcus murrayi</i>	WP_084542862.1	B	A
45		WP_051363537.1	B	B
46	<i>Deinococcus reticulitermitis</i>	WP_092263046.1	B	B
47	<i>Deinococcus wulumuqiensis</i>	WP_017870657.1	B	B
48	<i>Deinococcus</i> sp. Leaf326	WP_056297006.1	B	B
49	<i>Deinococcus phoenicis</i>	WP_034353069.1	B	B
50	<i>Deinococcus proteolyticus</i>	WP_013614672.1	B	B
51	<i>Deinococcus</i> sp. 17bor-2	WP_109826642.1	B	B
52	<i>Deinococcus</i> sp. NW-56	WP_104990527.1	B	B
53	<i>Deinococcus</i> sp. RL	KEF35131.1	B	B
54		WP_081851636.1	C	C
55	<i>Deinococcus</i> sp. YIM 77859	WP_034384462.1	B	A
56	<i>Desulfurococcus mucosus</i>	ADV64381.1	B	B
57		ADV64382.1	B	B
58		ADV64566.1	A	C
59	<i>Dictyoglomus turgidum</i>	YP_002352793.1	B	B
60		YP_002353123.1	B	B
61		YP_002352154.1	B	A
62	<i>Effusibacillus pohliae</i>	WP_018132070.1	A	C
63	<i>Fervidobacterium gondwanense</i>	WP_072759218.1	A	C
64	<i>Fervidobacterium islandicum</i>	ANW32328.1	B	B
65		ANW33413.1	B	B
66	<i>Fervidobacterium nodosum</i>	ABS60322.1	B	B
67		ABS61294.1	A	C
68	<i>Fervidobacterium pennivorans</i>	AFG34634.1	A	C
69		AFG35917.1	B	B
70		WP_064011782.1	B	B

71	<i>Geobacillus</i> sp.	WP_011232549.1	A	C
72	<i>Geobacillus stearothermophilus</i>	ALA69320.1	B	B
73		ALA71677.1	B	A
74		ALA71036.1	A	C
75		ALA70927.1	A	C
76		ALA69332.1	B	B
77	<i>Halococcus salifodinae</i>	WP_005045395.1	B	A
78		EMA49396.1	A	C
79		EMA52756.1	A	C
80	<i>Hydrogenivirga</i> sp. 128-5-R1-1	WP_008285887.1	B	B
81	<i>Hydrogenobacter hydrogenophilus</i>	WP_096602199.1	B	B
82		WP_096601840.1	A	C
83	<i>Hydrogenobacter thermophilus</i>	WP_012963417.1	A	C
84	<i>Hyperthermus butylicus</i>	AEM80367.1	A	C
85	<i>Kosmotoga arenicorallina</i>	WP_084251417.1	B	A
86	<i>Kosmotoga olearia</i>	ACR80305.1	A	C
87		ACR80819.1	A	C
88	<i>Marinitoga piezophila</i>	AEX84499.1	B	A
89		AEX85453.1	A	C
90		AEX85799.1	A	C
91	<i>Meiothermus cerbereus</i>	WP_027877060.1	B	B
92		WP_027878515.1	B	B
93	<i>Meiothermus chliarophilus</i>	WP_027892542.1	B	B
94		WP_051304156.1	A	C
95	<i>Meiothermus ruber</i>	ADD26908.1	B	B
96		WP_013013826.1	A	C
97		WP_013013825.1	A	C
98		WP_013012548.1	A	C
99		WP_027883570.1	B	B
100	<i>Meiothermus silvanus</i>	ADH63424.1	B	B
101		WP_013157023.1	B	B
102	<i>Meiothermus taiwanensis</i>	WP_027888084.1	B	B
103		WP_027888305.1	B	B

104	<i>Meiothermus timidus</i>	WP_018466213.1	B	A
105		WP_018465080.1	B	A
106		WP_018467688.1	A	C
107		WP_018467518.1	A	C
108		WP_018465603.1	B	A
109		WP_018466116.1	A	C
110		WP_018467797.1	A	C
111		WP_018467420.1	A	C
112		WP_018466214.1	A	C
113		WP_018465420.1	B	B
114	<i>Meiothermus rufus</i>	WP_036271046.1	B	B
115	<i>Mesotoga infera</i>	KUK90417.1	A	C
116	<i>Metallosphaera sedula</i>	ABP94905.1	B	B
117	<i>Methanocella conradii</i>	AFC99407.1	B	B
118		WP_014405429.1	A	C
119		AFD00405.1	A	C
120	<i>Methanococcoides methylutens</i>	WP_048194361.1	A	C
121		AKB84306.1	A	C
122	<i>Methanohalobium evestigatum</i>	ADI75005.1	B	A
123		WP_013193898.1	A	C
124	<i>Methanlobus tindarius</i>	WP_048135864.1	A	C
125		WP_048135649.1	A	C
126		ETA67773.1	A	C
127	<i>Methanosarcina siciliae</i>	WP_048179383.1	A	C
128		AKB37722.1	A	C
129		AKB35909.1	A	C
130	<i>Methanothermus fervidus</i>	ADP76907.1	A	C
131	<i>Petrotoga mobilis</i>	ABX30802.1	B	B
132		KUK15285.1	A	C
133	<i>Picrophilus torridus</i>	AAT44010.1	B	A
134		AAT43693.1	B	B
135	<i>Pseudonocardia thermophila</i>	WP_073456926.1	A	C

136	<i>Pyrococcus furiosus</i>	AAL80556.1	B	B
137		AAL81109.1	A	C
138		WP_014835302.1	A	C
139		AAL82045.1	A	C
140		AAL82122.1	A	C
141		AAL81285.1	A	C
142		AAL81790.1	B	B
143		AAL80346.1	B	A
144		AAL80703.1	B	A
145		AAL81901.1	B	B
146		AAL81913.1	B	B
147		AAL80587.1	B	A
148		AFN04628.1	B	B
149	<i>Pyrodictium occultum</i>	WP_083494490.1	A	C
150	<i>Rhodothermus marinus</i>	WP_041806336.1	B	A
151		WP_012844425.1	B	A
152		ACY47561.1	B	A
153		ACY48134.1	A	C
154		ACY47672.1	B	A
155		ACY48911.1	B	A
156		WP_012843092.1	B	A
157		WP_012843709.1	A	C
158		WP_012844300.1	A	C
159		ACY48106.1	B	A
160		WP_012844667.1	B	A
161		WP_012845116.1	B	A
162		WP_012844208.1	B	A
163		WP_012843855.1	B	A
164		ACY47289.1	B	B
165	<i>Slackia heliotrinireducens</i>	WP_012798605.1	A	C
166		ACV22623.1	A	C
167	<i>Staphylothermus marinus</i>	ABN70247.1	B	B
168		ABN69637.1	A	C
169		ABN69899.1	B	B

170	<i>Sulfolobus acidocaldarius</i>	AAV79446.1	A	C
171		WP_015385352.1	A	C
172		AAV80292.1	B	B
173	<i>Thermanaerothermophilus daxensis</i>	KPL83857.1	B	A
174	<i>Thermoanaerobacter</i> sp.	WP_004400108.1	A	C
175	<i>Thermoanaerobacter thermohydrosulfuricus</i>	WP_074592559.1	B	A
176		WP_074665888.1	A	C
177	<i>Thermoanaerobacter wiegelii</i>	AEM79256.1	B	B
178		AEM79130.1	A	C
179	<i>Thermoanaerobacterium xylanolyticum</i>	WP_013788008.1	A	C
180	<i>Thermobifida halotolerans</i>	WP_068689397.1	A	C
181		WP_068692137.1	A	C
182	<i>Thermococcus celer</i>	WP_088862065.1	A	C
183		ASI98087.1	A	C
184	<i>Thermococcus litoralis</i>	EHR78031.1	B	B
185	<i>Thermococcus profundus</i>	WP_088857759.1	B	B
186	<i>Thermocrinis minervae</i>	WP_079653666.1	B	B
187	<i>Thermocrinis ruber</i>	AHE95294.1	B	B
188	<i>Thermoflexus hugenholtzii</i>	WP_088571736.1	A	C
189	<i>Thermotoga lettingae</i>	ABV34293.1	B	B

190	<i>Thermotoga neapolitana</i>	WP_015919708.1	A	C
191		ACM22182.1	B	B
192		ACM22192.1	B	B
193		ACM22252.1	A	C
194		ACM22284.1	A	C
195		ACM22382.1	B	A
196		ACM22666.1	A	C
197		ACM22704.1	A	C
198		ACM22708.1	B	A
199		ACM22832.1	A	C
200		ACM22990.1	A	C
201		ACM23016.1	A	C
202		ACM23040.1	A	C
203		ACM23081.1	A	C
204		ACM23138.1	A	C
205		ACM23254.1	A	A
206		ACM23323.1	A	C
207		ACM23341.1	A	C
208		ACM23415.1	A	C
209		ACM23510.1	A	C
210		ACM23571.1	B	A
211		ACM23575.1	B	B
212		ACM23847.1	A	C
213		ACM23859.1	A	C
214		ACM23950.1	B	A
215		ACM23957.1	A	C
216		ACM24018.1	B	A
217	<i>Thermotoga petrophilia</i>	ABQ46309.1	B	B
218	<i>Thermus amyloliquefaciens</i>	WP_038055695.1	A	C
219	<i>Thermus filiformis</i>	WP_038062097.1	A	C
220	<i>Thermus thermophilus</i>	WP_096411270.1	A	C
221		AAS82270.1	B	B
222	<i>Truepera radiovictrix</i>	WP_013176662.1	A	C

Example 4: Analysis of Psicose Production Activity Through Multiple Enzymatic Reactions (Multiple Enzymes)

For the production of psicose from maltodextrin, seven enzymes were selected and reacted simultaneously (one-pot). As the seven enzymes, glucan phosphorylase, which degrades starch and converts it into glucose-1-phosphate, pullulanase, which debranches starch, 4- α -glucanotransferase, which increases the utilization of starch substrate, phosphoglucomutase, which converts glucose-1-phosphate into glucose-6-phosphate, glucose-6-phosphate isomerase, which converts glucose-6-phosphate into fructose-6-phosphate, psicose-6-phosphate-3-epimerase, which converts fructose-6-phosphate into psicose-6-phosphate, and enzymes of SEQ ID NOS: 1 to 222 capable of producing psicose from psicose-6-phosphate were prepared and used in an amount of 0.1 unit/mL. 5% (w/v) maltodextrin was added to 1 mM to 5 mM MgCl_2 and 10 mM to 50 mM sodium phosphate (pH 7.0), and the mixture was allowed to react at a temperature of 50°C for 12 hours. The reaction products were analyzed by HPLC using an Aminex HPX-87C column (Bio-RAD) at 80°C at a mobile phase flow rate of 0.6 mL/min and detected using a refractive index detector.

As a result, it was confirmed that psicose was produced from maltodextrin through the multiple enzymatic reactions.

While the present application has been described with reference to the particular illustrative embodiments, it will be understood by those skilled in the art to which the present application pertains that the present application may be embodied in other specific forms without departing from the technical spirit or essential characteristics of the present application. Therefore, the embodiments described above are considered to be illustrative in all respects and not restrictive. Furthermore, the scope of the present application is defined by the appended claims rather than the detailed description, and it should be understood that all modifications or variations derived from the meanings and scope of the present application and equivalents thereof are included in the scope of the appended claims.

[Deposition No.]

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Deposition Date: 20181114



BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To, CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
JUNG-GU, SEOUL 100-400
REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap26	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12390P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on November, 14, 2018 (date of the original deposit). ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November, 14, 2018



¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To: CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
JUNG-GU, SEOUL 100-400
REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap29	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12391P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
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Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjennae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November. 14. 2018.



¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

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03641: 서울시 서대문구 홍제2가길 45 유림빌딩 Tel: 02-391-0950, 392-0950 Fax: 02-392-2819

— KOREAN CULTURE CENTER OF MICROORGANISMS —

Yurim Bldg., 45, Hongjennae 2ga-gil, Seodaemun-gu, Seoul, 03641, Korea Tel: 82-2-391-0950, 392-0950 Fax: 82-2-392-2819



BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To, CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
JUNG-GU, SEOUL 100-400,
REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap53	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12392P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on November, 14, 2018 (date of the original deposit). ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November, 14, 2018.



¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page)



BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To: CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
JUNG-GU, SEOUL 100-400
REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap56	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12393P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
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Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjense-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November. 14. 2018.

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한국미생물보존센터

03641 서울시 서대문구 용저사(2가)길 45 유원빌딩 Tel: 02-391-0950, 396-0950 Fax: 02-392-2859

KOREAN CULTURE CENTER OF MICROORGANISMS

Seodaemun Bldg., 45, Hongjense-2ga-gil, Seodaemun-gu, Seoul, 03641, Korea Tel: 82-2-391-0950, 396-0950 Fax: 82-2-392-2859



BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To: CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
JUNG-GU, SEOUL 100-400
REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap60	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12394P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
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Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November. 14. 2018.

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INTERNATIONAL FORM

To: CJ CheilJedang Corporation
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330, DONGHO-RO,
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REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
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INTERNATIONAL DEPOSITARY AUTHORITY
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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap70	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12395P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
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Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November. 14, 2018.



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한국미생물보존센터

서울특별시 서대문구 홍제1로27길 45 유원빌딩

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45, Hongjeonae-2ga-gil, Seodaemun-gu, Seoul, 03641, Korea

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To, CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
JUNG-GU, SEOUL 100-400
REPUBLIC OF KOREA

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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap76	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12396P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
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Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjense-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November, 14, 2018.

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한국미생물보존센터

03641 서울시 서대문구 용자내2가길 45 유림빌딩 Tel: 02-391-0950, 396-0950 Fax: 02-392-2659

KOREAN CULTURE CENTER OF MICROORGANISMS

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Tel: 82-2-391-0950, 396-0950 Fax: 82-2-392-2659



BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To: CJ CheilJedang Corporation
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330, DONGHO-RO,
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REPUBLIC OF KOREA

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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap80	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12397P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
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V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjenae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November. 14. 2018.

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To: CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
JUNG-GU, SEOUL 100-400
REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap81	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12398P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
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Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November. 14. 2018.

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한국미생물보존센터

03641 서울시 서대문구 순자나2가길 45 옥원빌딩 Tel: 02-391-0950, 396-0999 Fax: 02-392-3859

KOREAN CULTURE CENTER OF MICROORGANISMS

Yurim Bldg., 45, Hongjeonae 2ga-gil, Seodaemun-gu, Seoul, 03641, Korea Tel: 82-2-391-0950, 396-0999 Fax: 82-2-392-3859



BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To: CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
JUNG-GU, SEOUL 100-400
REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap116	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12399P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on November. 14. 2018 (date of the original deposit). ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yoon B/D 45, Hongjennae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November. 14. 2018.



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한국미생물보존센터

03641 서울시 서대문구 홍제2가길 45 유원빌딩 Tel: 02-391-0950, 398-0950 Fax: 02-392-2959

KOREAN CULTURE CENTER OF MICROORGANISMS

Yoon Bldg., 45, Hongjennae-2ga-gil, Seodaemun-gu, Seoul, 03641, Korea Tel: 82-2-391-0950, 398-0950 Fax: 82-2-392-2959



BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

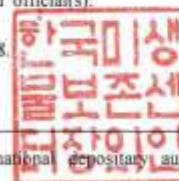
INTERNATIONAL FORM

To: CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
JUNG-GU, SEOUL 100-400
REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
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INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap117	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12400P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
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Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November. 14, 2018.

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03641 서울시 서대문구 홍제2가길 45 휴원빌딩 Tel: 02-391-0950, 398-0950 Fax: 02-392-0959

KOREAN CULTURE CENTER OF MICROORGANISMS

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BUDAPEST TREATY ON THE INTERNATIONAL
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INTERNATIONAL FORM

To, CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
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REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap131	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12401P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
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Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November. 14. 2018

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— 한국미생물보존센터 —

03641 서울시 서대문구 홍제2가길 45 유림빌딩 Tel: 02-381-0950, 396-0950 Fax: 02-392-2869

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BUDAPEST TREATY ON THE INTERNATIONAL
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INTERNATIONAL FORM

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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap134	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12402P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
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Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjenseo-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November. 14. 2018.



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03641 서울시 서대문구 홍제1로2가길 45-유원빌딩 Tel: 02-391-0950, 296-0950 Fax: 02-392-2958

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INTERNATIONAL FORM

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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap145	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCCM12403P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
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The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November, 14, 2018.



¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page)



BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To: CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
JUNG-GU, SEOUL 100-400
REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap167	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12404P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on November. 14, 2018 (date of the original deposit). ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim D/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s). Date: November. 14, 2018.



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Form BP/4 (sole page)

한국미생물보존센터

45, Hongjeonae-2ga-gil, Seodaemun-gu, Seoul, 03641, Korea

03641 서울시 서대문구 홍제42가길 45 우정빌딩 TEL 02-331-0950, 336-0350 FAX 02-332-2559

KOREAN CULTURE CENTER OF MICROORGANISMS

45, Hongjeonae-2ga-gil, Seodaemun-gu, Seoul, 03641, Korea

Yurim Bldg., 45, Hongjeonae-2ga-gil, Seodaemun-gu, Seoul, 03641, Korea TEL 82-2-331-0950, 336-0350 FAX 82-2-332-2559



BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To: CJ CheilJedang Corporation
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RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
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INTERNATIONAL DEPOSITARY AUTHORITY
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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap185	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12405P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation. (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on November. 14. 2018 (date of the original deposit). ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November. 14. 2018.

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.



Form BP/4 (sole page)

한국미생물보존센터

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Yurim Bldg., 45, Hongjeonae 2ga-gil, Seodaemun-gu, Seoul, 03641, Korea Tel: 82-2-391-0950, 396-0950 Fax: 82-2-392-2859



BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To: CJ CheilJedang Corporation
CJ CHEILJEDANG CENTER,
330, DONGHO-RO,
JUNG-GU, SEOUL 100-400
REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap186	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12406P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on November, 14, 2018 (date of the original deposit). ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjeda-e2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November, 14, 2018.

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.



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한국미생물보존센터

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BUDAPEST TREATY ON THE INTERNATIONAL
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FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To: CJ CheilJedang Corporation
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REPUBLIC OF KOREA

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> BL21(DE3)/pET-CJ-ap191	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM12407P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on November. 14. 2018 (date of the original deposit). ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03641 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: November. 14. 2018.

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.



Form BP/4 (sole page)

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CLAIMS

1. A composition for producing psicose, comprising a psicose-6-phosphate phosphatase consisting of any one of amino acid sequences of SEQ ID NOS: 1, 6, 9, 12 to 14, 17, 18, 20 to 23, 25, 26, 28, 29, 37 to 53, 55 to 57, 59 to 61, 64 to 66, 69, 70, 72, 73, 76, 77, 80, 81, 85, 88, 91, 93, 95, 99, 100, 102, 104, 105, 108, 114, 116, 117, 122, 131, 133, 134, 136, 142 to 148, 150 to 152, 154 to 156, 159 to 164, 167, 169, 172, 173, 175, 177, 184 to 187, 189, 191, 192, 195, 198, 205, 210, 211, 214, 216, 217, and 221, a microorganism expressing the same, or a culture of the microorganism.
2. The composition of claim 1, wherein the psicose-6-phosphate phosphatase consists of any one of amino acid sequences of SEQ ID NOS: 1, 6, 9, 12, 26, 29, 38 to 43, 45 to 53, 56, 57, 59, 60, 64 to 66, 69, 70, 72, 76, 80, 81, 91, 93, 95, 99, 100, 102, 114, 116, 117, 131, 134, 136, 142, 145, 146, 148, 164, 167, 169, 172, 177, 184 to 187, 189, 191, 192, 211, 217, and 221.
3. The composition of claim 1, wherein the psicose-6-phosphate phosphatase exhibits an activity selective to psicose-6-phosphate.
4. The composition of claim 1, wherein the composition further comprises a sugar phosphate.
5. A method for producing psicose, comprising converting psicose-6-phosphate into psicose by bringing psicose-6-phosphate into contact with a psicose-6-phosphate phosphatase consisting of any one of amino acid sequences of SEQ ID NOS: 1, 6, 9, 12 to 14, 17, 18, 20 to 23, 25, 26, 28, 29, 37 to 53, 55 to 57, 59 to 61, 64 to 66, 69, 70, 72, 73, 76, 77, 80, 81, 85, 88, 91, 93, 95, 99, 100, 102, 104, 105, 108, 114, 116, 117, 122, 131, 133, 134, 136, 142 to 148, 150 to 152, 154 to 156, 159 to 164, 167, 169, 172, 173, 175, 177, 184 to 187, 189, 191, 192, 195, 198, 205, 210, 211, 214, 216, 217, and 221, a microorganism expressing the same, or a culture of the microorganism.
6. The method of claim 5, wherein the method further comprises converting fructose-6-phosphate into psicose-6-phosphate by bringing fructose-6-phosphate into contact with fructose-6-phosphate-3-epimerase, a microorganism expressing the same, or a culture of the microorganism, prior to the converting psicose-6-phosphate into

psicose.

7. The method of claim 6, wherein the method further comprises converting glucose-6-phosphate into fructose-6-phosphate by bringing glucose-6-phosphate into contact with glucose-6-phosphate-isomerase, a microorganism expressing the same, or a culture of the microorganism, prior to the converting fructose-6-phosphate into psicose-6-phosphate.
8. The method of claim 7, wherein the method further comprises converting glucose-1-phosphate into glucose-6-phosphate by bringing glucose-1-phosphate into contact with phosphoglucomutase, a microorganism expressing the same, or a culture of the microorganism, prior to the converting glucose-6-phosphate into fructose-6-phosphate.
9. The method of claim 7, wherein the method further comprises converting glucose into glucose-6-phosphate by bringing glucose into contact with polyphosphate glucokinase, a microorganism expressing the same, or a culture of the microorganism, and polyphosphate, prior to the converting glucose-6-phosphate into fructose-6-phosphate.
10. The method of claim 8, wherein the method further comprises converting starch, maltodextrin, sucrose, or a combination thereof into glucose-1-phosphate by bringing starch, maltodextrin, sucrose, or a combination thereof into contact with α -glucan phosphorylase, starch phosphorylase, maltodextrin phosphorylase, or sucrose phosphorylase; a microorganism expressing the same; or a culture of the microorganism, and phosphate, prior to the converting glucose-1-phosphate into glucose-6-phosphate.
11. The method of claim 10, wherein the converting starch, maltodextrin, sucrose, or a combination thereof into glucose-1-phosphate further comprises converting starch, maltodextrin, sucrose, or a combination thereof into a maltooligosaccharide or glucose by further comprising α -amylase, pullulanase, isoamylase, α -glucanotransferase, glucoamylase, or sucrase; a microorganism expressing the same; or a culture of the microorganism.
12. A method for producing psicose, comprising bringing starch, maltodextrin, sucrose, or

- a combination thereof and phosphate into contact with (a) psicose-6-phosphate phosphatase; fructose-6-phosphate-3-epimerase; glucose-6-phosphate-isomerase; phosphoglucomutase or glucokinase; and α -glucan phosphorylase, starch phosphorylase, maltodextrin phosphorylase, sucrose phosphorylase, α -amylase, pullulanase, isoamylase, glucoamylase, α -glucanotransferase, polyphosphate glucokinase, or sucrase; or (b) a microorganism expressing the enzymes of item (a) or a culture of the microorganism, wherein the psicose-6-phosphate phosphatase consists of any one of amino acid sequences of SEQ ID NOS: 1, 6, 9, 12 to 14, 17, 18, 20 to 23, 25, 26, 28, 29, 37 to 53, 55 to 57, 59 to 61, 64 to 66, 69, 70, 72, 73, 76, 77, 80, 81, 85, 88, 91, 93, 95, 99, 100, 102, 104, 105, 108, 114, 116, 117, 122, 131, 133, 134, 136, 142 to 148, 150 to 152, 154 to 156, 159 to 164, 167, 169, 172, 173, 175, 177, 184 to 187, 189, 191, 192, 195, 198, 205, 210, 211, 214, 216, 217, and 221.
13. The method of any one of claims 6 to 12, the contact is performed at a pH of 5.0 to 9.0, at a temperature of 40°C to 80°C, and/or for 2 hours to 24 hours or 120 hours.
14. The method of claim 5, wherein the psicose-6-phosphate phosphatase consists of any one of amino acid sequences of SEQ ID NOS: 1, 6, 9, 12, 26, 29, 38 to 43, 45 to 53, 56, 57, 59, 60, 64 to 66, 69, 70, 72, 76, 80, 81, 91, 93, 95, 99, 100, 102, 114, 116, 117, 131, 134, 136, 142, 145, 146, 148, 164, 167, 169, 172, 177, 184 to 187, 189, 191, 192, 211, 217, and 221.
15. The method of claim 5, wherein the psicose-6-phosphate phosphatase exhibits an activity selective to psicose-6-phosphate.