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(54) **ARABINOSE ISOMERASE EXPRESSED FROM CORYNEBACTERIUM GENUS AND TAGATOSE MANUFACTURING METHOD BY USING IT**

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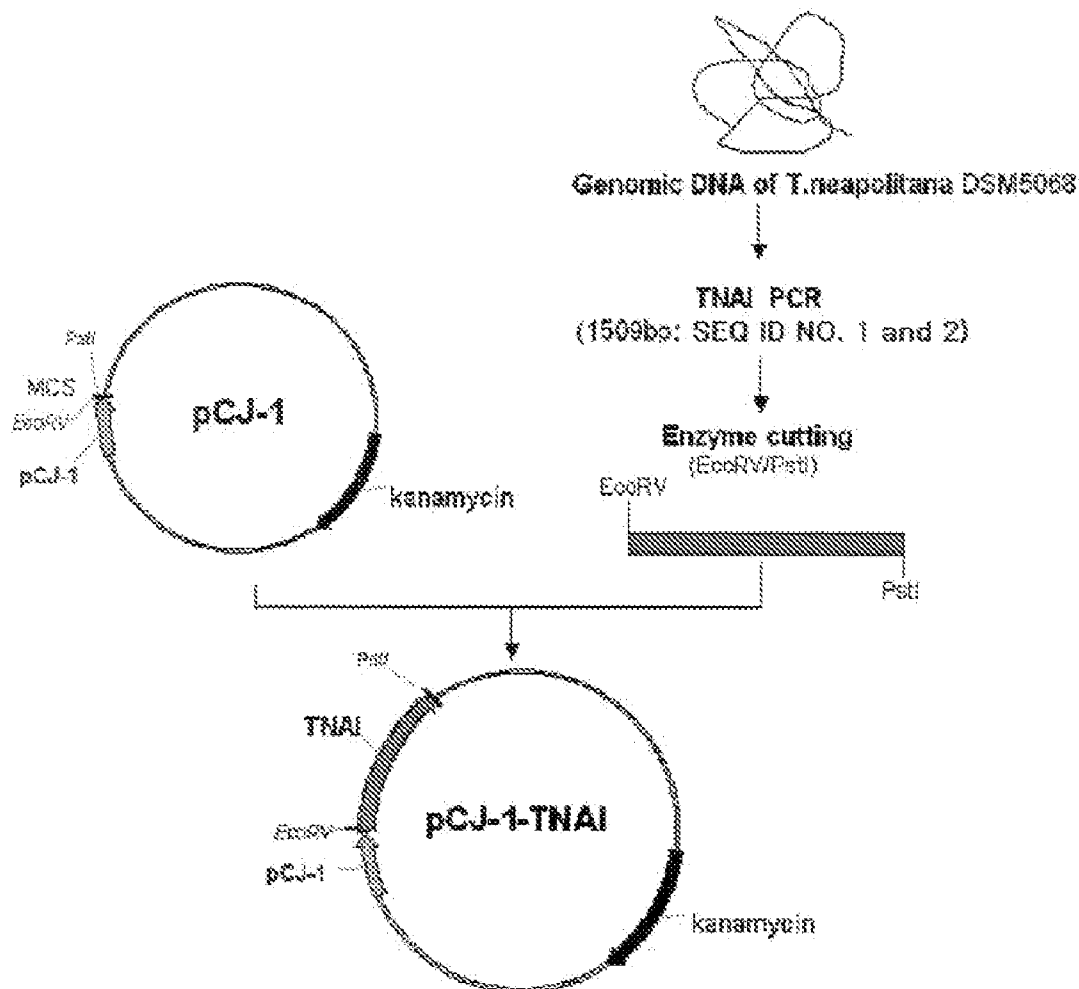
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ABSTRACT

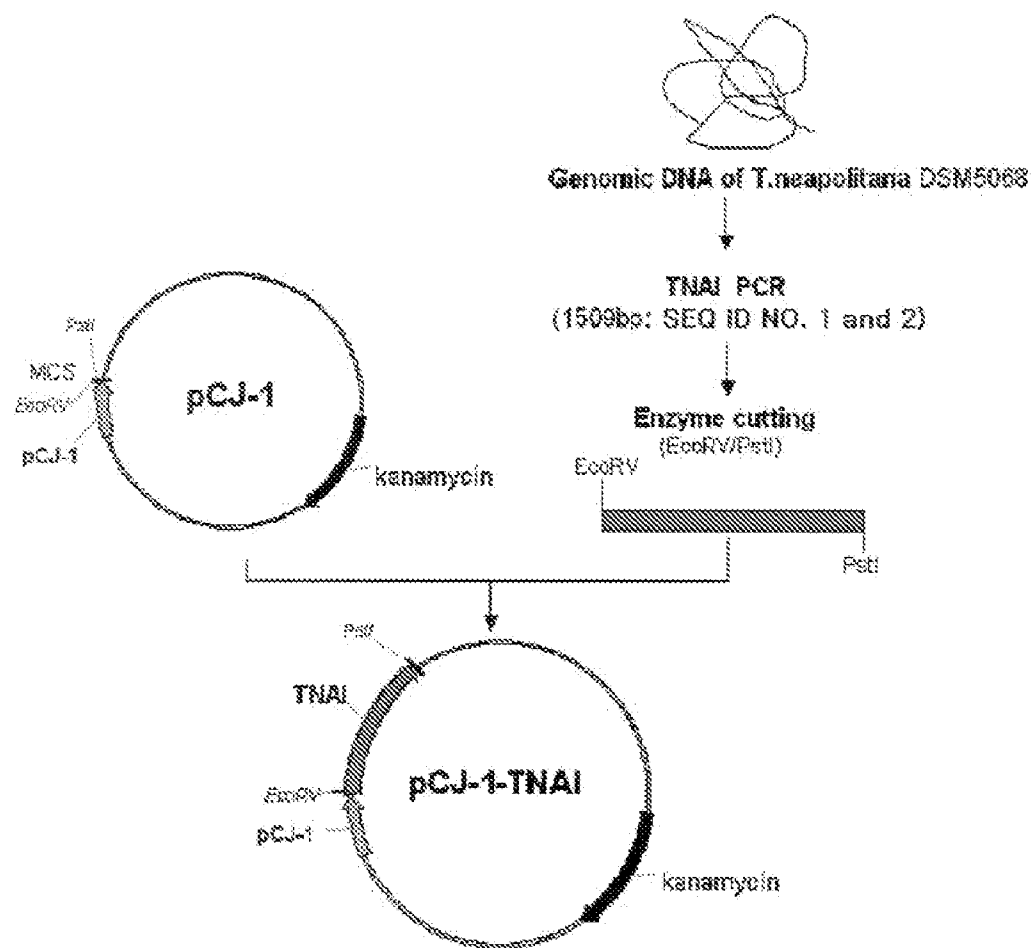
The present invention relates to a thermophilic arabinose isomerase and a method of manufacturing tagatose using the same, and more precisely, a gene encoding arabinose isomerase originating from the thermophilic *Thermotoga neapolitana* DSM 5068, a recombinant expression vector containing the gene, a method of preparing a food grade thermophilic arabinose isomerase from the recombinant GRAS (Generally Recognized As Safe) strain transformed with the said expression vector, and a method of preparing tagatose from galactose using the said enzyme.

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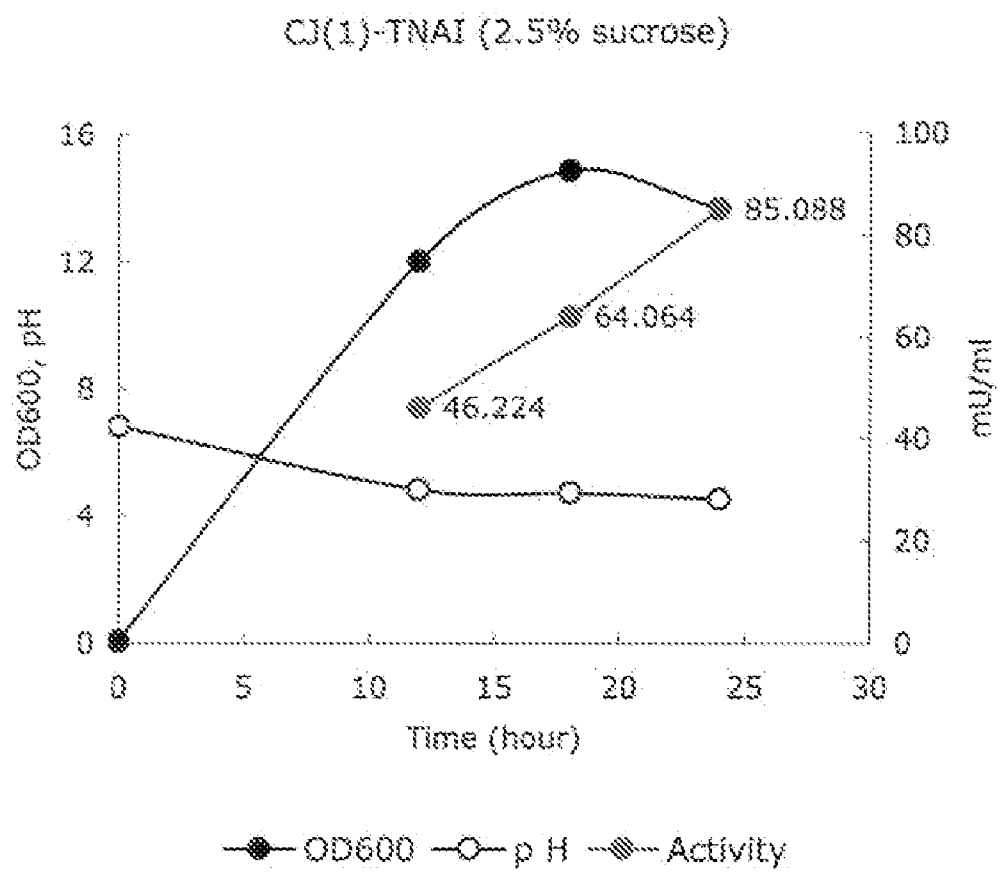
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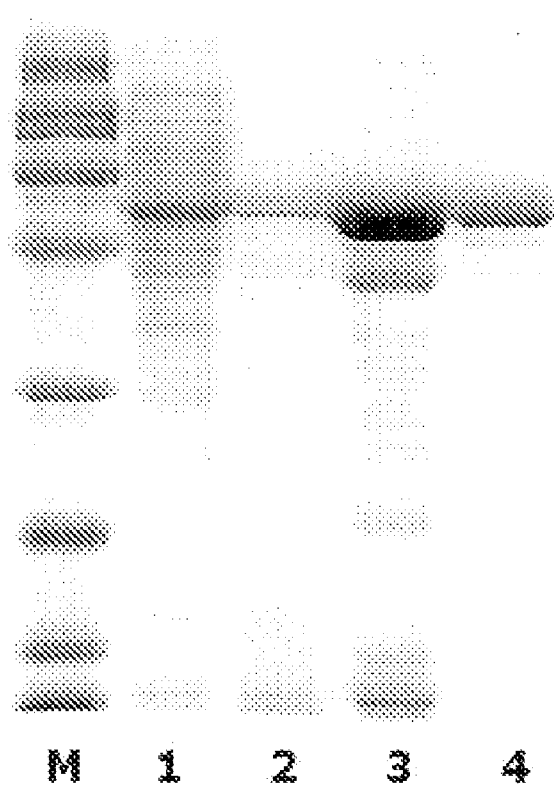
【Figure 1】



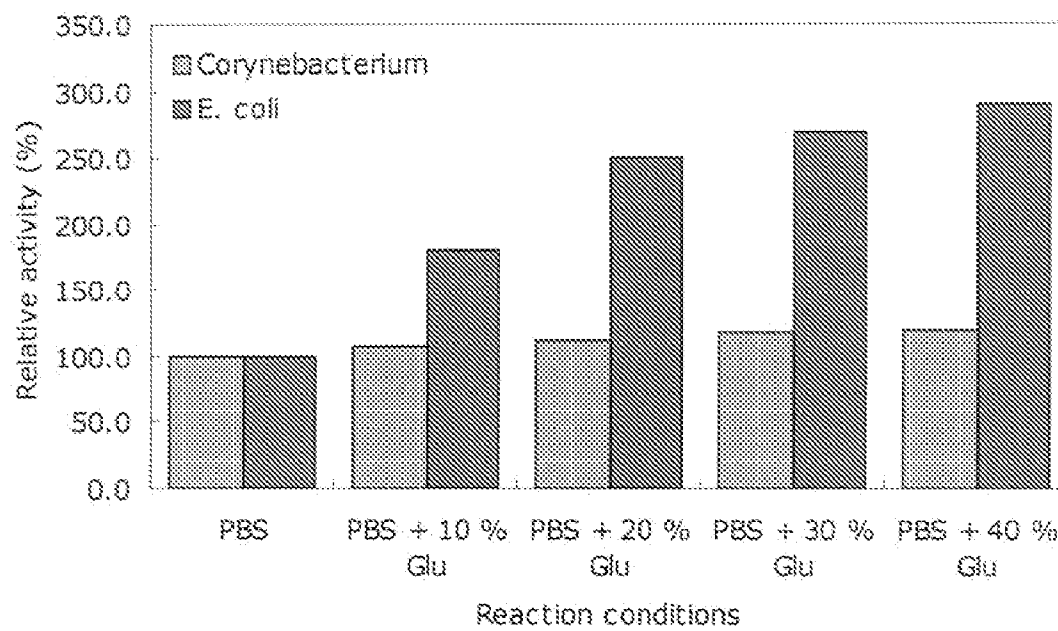
【Figure 2】



【Figure 3】



【Figure 4】



**ARABINOSE ISOMERASE EXPRESSED
FROM CORYNEBACTERIUM GENUS AND
TAGATOSE MANUFACTURING METHOD BY
USING IT**

TECHNICAL FIELD

[0001] The present invention relates to an arabinose isomerase gene expressed in a *Corynebacterium* sp. strain and a method of preparing tagatose using the same, and more particularly, the present invention provides a gene encoding arabinose isomerase originating from *Thermotoga neapolitana* DSM 5068, a recombinant expression vector containing the gene, a method of preparing a thermophilic arabinose isomerase by expressing the gene in a food grade GRAS *Corynebacterium* sp. strain transfected with the recombinant expression vector, and a method of preparing tagatose from galactose using the same.

BACKGROUND ART

[0002] With the increasing interest in well-being or a healthy life, tagatose has been proposed as an alternative to sugar as it has less side effects and sugar is one of the major factors causing various adult diseases. Tagatose is the isomer of galactose and is known to have fructose-like physiochemical properties. Tagatose is a natural low-calorie sugar, and has recently been approved by the FDA in the USA as GRAS (Generally Recognized As Safe), so it is now allowed to be added as a sweetener to foods, beverages, health foods, diet additives, etc.

[0003] GRAS indicates a substance that is generally recognized as safe, which is judged by specialized people having enough experience and skills through scientific procedure and examination under the indicated conditions and purpose of use. GRAS is a unique system used only in the USA to evaluate the safety of foods and food chemical substances (under certain conditions) but it is recognized worldwide.

[0004] Tagatose is produced by either isomerization, which is a chemical method using a catalyst to produce an isomer, of galactose or a biological method using isomerase to convert galactose enzymatically.

[0005] One of the biological methods well-known to those in the art is to convert aldose or aldose derivatives into ketose or ketose derivatives by using an enzyme. The isomerizations of galactose to tagatose by using arabinose isomerase is generally carried out thermodynamically at high temperature and exhibits a proportionally high conversion rate. Therefore, developing an enzyme that works stably at high temperature and a method of preparing tagatose using the same are key techniques for the industrial application thereof based on the biological conversion of tagatose using an isomerase. By screening thermophilic bacteria derived arabinose isomerases, an industrially applicable thermophilic isomerase has been tried, and efforts have also been made by many research teams to establish an isomerization process using the same.

[0006] In Korea, an enzymatic isomerization method using arabinose isomerase has been developed by Tong Yang Confectionary Corp. According to the method, the arabinose isomerase derived from *E. coli* was homogeneously expresses in *E. coli* by recombinant technology. This recombinant isomerase was reacted at 30° C. for 24 hours to convert galactose into tagatose, and at this time the conversion rate was 25%, indicating that both thermostability and conversion

yield were very low (Korean Patent Application No. 99-16118). Professor Oh, Deok-keun and his colleagues (Sejong University) succeeded in heterogeneous expression of arabinose isomerase originating from *Geobacillus stearothermophilus* in *E. coli* and based on that they proposed an isomerization procedure at high temperature to convert galactose into tagatose. The present inventors succeeded in cloning a novel arabinose isomerase gene from *Thermotoga neapolitana* and producing it in *E. coli*, and further developed a method of manufacturing tagatose at hyper-thermophilic condition using the enzyme (Korean Patent No. 10-0443865).

[0007] the production of tagatose using a derived arabinose isomerase derived from thermophiles still depends on the method of using a recombinant enzyme mass-expressed in recombinant *E. coli* or the isomerization of galactose to tagatose using a host containing the recombinant enzyme. However, the biological production of tagatose using recombinant *E. coli* is not appropriate for the production of tagatose as a food ingredient. To produce tagatose as a food additive, arabinose isomerase expressed in a host which is GRAS microorganism appropriate for the mass-production of the same is essential.

[0008] *Corynebacterium* is an industrial microorganism that has been used for the production of chemical compounds applicable to a variety of fields including L-lysine, L-threonine and various nucleic acid containing feeds, medical supplies and foods. The *Corynebacterium* is a GRAS (generally Recognized As Safe) strain, which favors gene manipulation and mass production. In addition, this strain is highly stable under the various process conditions and has a comparatively stable cell membrane, compared with other bacteria. This, this strain remains stable even under high osmotic pressure generated by a high concentration of galactose.

[0009] The present inventors found that thermophilic arabinose isomerase originating from the hyperthermophilic *Thermotoga neapolitana* is overexpressed as an active form in a GRAS *Corynebacterium* sp. strain, and further completed this invention by developing a novel art to induce tagatose isomerization from the high concentration of galactose by using the expressed recombinant enzymes from *Corynebacterium* species.

DISCLOSURE OF THE INVENTION

[0010] It is an object of the present invention to provide a method of preparing GRAS strains expressing arabinose isomerase stably in their cells under the conditions of high temperature and high concentration of galactose by taking advantage of mass-production of the recombinant enzyme in the *Corynebacterium* sp. strain.

[0011] To overcome the limits of the convention method in which an enzyme could be expressed only in *E. coli*, it is also an object of the present invention to provide a method of preparing tagatose in which arabinose isomerase originating from *Thermotoga* is expressed as an active form in a GRAS *Corynebacterium* strain, and accordingly food grade tagatose is generated from galactose using the expressed recombinant enzyme or hosts containing the same.

[0012] The above object and other objects of the present invention can be achieved by the following embodiments of the present invention.

[0013] Hereinafter, the present invention is described in detail.

[0014] The present invention provides a method for producing a recombinant enzyme by introducing an arabinose

isomerase gene from a microorganism which is very difficult to obtain from a direct cell culture, because the optimum growth conditions for the microorganism is at least 80° C. or are anaerobic conditions, into a food grade host and a method of producing tagatose from galactose using the same.

[0015] The arabinose isomerase preferably originates from *Thermotoga* sp., *Thermus* sp. or *Sulforobus* sp. strain whose optimum reaction temperature is in the range of 70~90° C.

[0016] The arabinose isomerase gene of the present invention preferably originates from a hyperthermophile and more preferably from the hyperthermophile *Thermotoga neapolitana* DSM 5068, which has the amino acid sequence represented by SEQ. ID. NO: 3.

[0017] The arabinose isomerase gene of the present invention can be modified by those in the art using any conventional mutagenesis method such as directed evolution and site-directed mutagenesis. Thus, any host cells that have a certain level of homology with a GRAS host, for example at least 70% but preferably at least 80% and more preferably at least 90% homology with a GRAS host, and a recombinant enzyme that is expressed as an active form in the host and any host cells containing the enzyme are all included in the criteria of the present invention.

[0018] The present invention also provides a vector containing a gene encoding the arabinose isomerase of the present invention. The vector of the present invention is a typical vector for cloning or gene expression. The vector is not limited to a specific one and any vector known to those in the art is acceptable.

[0019] In the present invention, a promoter which is active in *Corynebacterium* was used as the vector to express a thermophilic isomerase as an active form. The promoter sequence used for the gene expression in the *Corynebacterium* has not been identified unlike other promoters used in industrial microorganisms such as *E. coli* or *Bacillus subtilis*. Herein, a strong promoter that originated from *Corynebacterium*, a popular industrial microorganism, and is able to be expressed in *E. coli* has been developed. Tac promoter is known as being one of the strongest promoters. Tac promoter is prepared by fusion of a sequence of the -35 region of the tryptophane operon promoter of *E. coli* with a sequence of the -10 region of the lactose operon promoter of *E. coli*. The promoter composed of CJ-1~CJ-7 in the present invention was confirmed to be more effective in expressing a target gene in *Corynebacterium* sp. bacteria cells than tac promoter (Korean patent Publication No. 10-2006-0068505). The *Corynebacterium* promoter of the present invention exhibits promoter activity not only in *Corynebacterium* sp. microorganisms, but also in *Escherichia* sp. bacteria and *E. coli* cells. In particular, the promoter of the present invention exhibits promoter activity in *Escherichia* sp. bacteria cells at least two times higher than tac promoter does.

[0020] The food grade strain, which means a GRAS strain, of the present invention includes *Corynebacterium*, and more specifically *Corynebacterium glutamicum* KCTC 10302.

[0021] According to an embodiment of the present invention, a recombinant strain was prepared by transforming *Corynebacterium* with the vector, which was then cultured to provide food grade arabinose isomerase. The culture medium and conditions depended on the kind of host.

[0022] The present invention provides a recombinant arabinose isomerase and a method of preparing tagatose from galactose by immobilizing host cells containing the recombinant enzyme.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The application of the preferred embodiments of the present invention is best understood with reference to the accompanying drawings, wherein:

[0024] FIG. 1 is a schematic diagram illustrating the construction of the recombinant expression vectors pCJ-1-TNAI and pCJ-7-TNAI containing a gene encoding the thermophilic arabinose isomerase originating from the *Thermotoga neapolitana* DSM 5068 strain,

[0025] FIG. 2 is a graph illustrating the time dependent culture growth of the recombinant *Corynebacterium* strain. Particularly, the recombinant *Corynebacterium* was cultured in an optimum medium, at 30° C. for 24 hours.

[0026] FIG. 3 is a photograph illustrating the results of SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) of the total protein expression patterns observed in crude enzyme solutions of each step of separation and purification of the arabinose isomerase expressed from the recombinant *Corynebacterium*. Each protein band indicates as follows:

[0027] M: wide range protein marker (Bio-Rad, U.S.A)

[0028] 1: crude enzyme solution

[0029] 2: heat treated enzyme supernatant

[0030] 3. enzyme fragment after anion exchange chromatography (HiTrap Q column, GE Healthcare, U.S.A)

[0031] FIG. 4 is a graph illustrating the amount of arabinose isomerase. The recombinant *Corynebacterium* and *E. coli* containing the arabinose isomerase expressed as an active form therein were reacted in PBS (phosphate-buffered saline) containing different galactose concentrations of 0, 10, 209, 30 and 40% for 2 hours at high temperature (70° C.) with stirring, and then the level of enzyme expression in the buffer was measured.

BEST MODE FOR CARRYING OUT THE INVENTION

[0032] Practical and presently preferred embodiments of the present invention are illustrated as shown in the following examples.

[0033] However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

EXAMPLES

[0034] In the preferred embodiment of the present invention, a gene encoding thermophilic arabinose isomerase originating from the hyperthermophile *Thermotoga neapolitana* DSM 5068 was inserted into pCJ-1 and pCJ-7 (*E. coli*~*Corynebacterium* shuttle vector, Korean patent Publication No. 10-2006-0068505). *Corynebacterium glutamicum* KCTC 13032 was transfected with the above vectors, in which the said protein was finally over-expressed. The recombinant strains *Corynebacterium glutamicum* CJ-1-TNAI (KCCM10786P) and CJ-7-TNAI (KCCM10787P) were cultured and cell extracts were obtained from each stage of the cell culture. The production activity of tagatose was measured, that is the amount of active recombinant protein

was measured stage by stage. The optimally expressed resultant culture was separated and purified by cell lysis, heat treatment, ion exchange resin and gel chromatography. Finally, the amino acid sequence of the amino-terminal was examined and the production activity of tagatose was confirmed by measurement of the protein activity.

Example 1

Cloning of the Arabinose Isomerase

[0035] *Thermotoga neapolitana* DSM 5068 was cultured under anaerobic conditions. Centrifugation was performed at 8,000×g for 10 minutes to recover the cultured cells. Genomic DNA was extracted from the obtained cells by using a Cell culture DNA Midi Kit (Qiagen, U.S.A.). Polymerase Chain Reaction (PCR) was performed with the genomic DNA by using oligonucleotides 5'-CCCGA TATCATGATCGATCTCAAACAGTATGAG-3' (SEQ. ID. NO: 1) and 5'-TGCAC-TGCAGTCATCTTTTAAAAGTCCCC-3' (SEQ. ID. NO: 2) with the insertion of EcoRV and PstI restriction enzyme site sequences as primers. PCR product was obtained by amplifying the 1509 bp DNA containing the *Thermotogas neapolitana* arabinose isomerase gene. To mass-produce the arabinose isomerase encoded by the amplified gene, two of the *Corynebacterium* sp. derived vectors exhibiting excellent protein overexpressing capacity were selected. The vectors were introduced into *E. coli* DH5alpha, which were deposited at the Korean Culture Center of Microorganisms (KCCM), an international depository authority (IDA), on Nov. 6, 2004 (Accession Nos. KCCM-10611 and KCCM-10617) (Table 1).

TABLE 1

Promoter	Vector	Accession No.	Derived Protein
pCJ1	pECCG117	KCCM-10611	Heat shock protein hsp60
pCJ7	pECCG117	KCCM-10617	Manganese superoxide dismutase

[0036] The PCR product digested with restriction enzymes EcoRV and PstI was inserted into the shuttle vectors pCJ-1 and pCJ-7, which were digested with the same enzymes, leading to the construction of the recombinant expression vectors pCJ-1-TNAI and pCJ-7-TNAI (see FIG. 1). *Corynebacterium glutamicum* KCTC 13032 was transfected with the recombinant expression vectors pCJ-1-TNAI and pCJ-7-TNAI to prepare recombinant strains, which were named '*Corynebacterium glutamicum* CJ-1-TNAI' and '*Corynebacterium glutamicum* CJ-7-TNAI'. The recombinant strains were deposited at the Korean Culture Center of Microorganisms (KCCM), an International Depository Authority (IDA), addresses at #361-221, Hongje 1-Dong, Secodaemun-Gu, Seoul, Korea, on Oct. 18, 2006 (Accession Nos: KCCM10786P and KCCM10787P).

Example 2

[0037] Expression of the Arabinose Isomerase in *Corynebacterium* the recombinant strains *Corynebacterium glutamicum* CJ-1-TNAI and *Corynebacterium glutamicum*

CJ-7-TNAI (Accession Nos: KCCM10786P and KCCM10787P) prepared in Example 1 were inoculated in MB medium (Bacto-trypton 10 g/L, bacto-yeast extract 5 g/L, NaCl 10 g/L, Soytone 5 g/L) containing 10 µg/Me of kanamycin at the concentration of OD₆₀₀=0.1, followed by culture at 30° C. for 24 hours to induce expression of the recombinant arabinose isomerase. To measure the enzyme activity of the expressed arabinose isomerase, the culture solution was centrifuged at 8,000×g for 10 minutes and cells were recovered. The cells were resuspended in 50 mM Tris-HCl (pH 7.0) buffer, followed by ultrasonification to lyse the cells. The supernatant was obtained as a crude enzyme solution, with which galactose isomerization was performed. Particularly, for the isomerization, 100 µl of an enzyme solution containing 40 mM of galactose as a substrate was mixed with 1 ml of a reaction buffer (50 mM Tris-HCl, pH 7.0). At that time, 5 mM of MnCl₂ and 1 mM of CoCl₂ were added to the reaction mixture. The crude enzyme reaction mixture was reacted at 60+ C. for 20 minutes. The activity of the crude enzyme was measured by the cystein-carbazol-sulfuric acid method (Dische, Z., and E. Borenfereund, A New Spectrophotometric Method for the Detection and Determination of Keto Sugars and Trioses, *J. Biol. Chem.*, 192:583-587, 1951). The protein contained in the crude enzyme solution was quantified with a Bradford assay kit (Biorad, U.S.A.). As a result, the isomerase activity was 2,050 (mg-tagatose/mg-protein-h), indicating that the product of galactose isomerization, tagatose, was successfully generated.

Example 3

Optimization of the Culture Condition for Mass-Production of the Recombinant Strain

[0038] The recombinant strains prepared in Example 2 were inoculated in MB medium (Bacto-trypton 10 g/L, bacto-yeast extract 5 g/L, NaCl 10 g/L, Soytone 5 g/L) containing 10 µg/Me of kanamycin at the primary concentration of OD₆₀₀=0.6. The growth of the strains in two basic media for the culture of *Corynebacterium*, MB medium (Bacto-trypton 10 g/L, bacto-yeast extract 5 g/L, NaCl 2.5 g/L, Beef extract 5 g/L), was investigated. Temperature dependent (25° C., 30° C., 37° C.), pH dependent, glucose (carbon source) and sucrose concentration dependent growths in the two media were compared. In addition, the growths under the various conditions and the expression levels of the enzyme thereby were measured every hour to judge the optimum expression condition for mass-production of the recombinant arabinose isomerase (Tables 2 and 3).

TABLE 2

	Modified medium	MB medium				
		Aerobic	Stationary	25° C.	30° C.	37° C.
OD ₆₀₀	10.1	9.4	5.56	8.88	10.1	4.44
pH	7.5	7.6	7.8	7.4	7.5	7.5
Activity (mU/ml)	60.907	56.09	34.2	58.783	60.907	29.054

TABLE 3

	Sucrose					Glucose			
	0%	2.5%	5%	7.5%	10%	2.5%	5%	7.5%	10%
OD ₆₀₀	10.1	13.6	12.72	14.86	12.94	14.7	14.32	10.96	4.36
pH	7.5	4.5	4.7	4.7	4.7	4.5	4.5	4.7	4.7
Activity (mU/ml)	60.907	85.088	82.160	79.104	73.708	67.935	58.896	58.649	42.441

[0039] To measure the enzyme activity of the recombinant arabinose isomerase, the enzyme was treated and quantified in the same manner as described in Example 2. When cells were cultured at 30% under aerobic conditions with the addition of 2.5% sucrose, tagatose, the product of galactose isomerization, showed approximately 85.1 mU/ml of enzyme activity which is 1.4 times higher than that observed in the standard culture (60.9 mU/ml), suggesting that the enzyme activity increased with the increase in the growth of the cells. The cell growth results in optimum medium are shown in FIG. 2.

Example 4

Separation and Purification of the Recombinant Arabinose Isomerase and Confirmation of the Expression of Active Protein by N-terminal Amino Acid Sequencing

[0040] 2 L culture of the recombinant strain was performed under the optimum culture conditions determined in the above Example 3. The culture solution was centrifuged at 8000×g for 10 minutes and cells were recovered. The cells were resuspended in 50 mM Tris-HCl (pH 7.0) buffer, and used for the protein purification. The cell suspension progressed to cell lysis using a high pressure cell homogenizer T-series (4.0 kW; constant systems, UK), followed by heat-treatment at 80° C. for 20 minutes. Centrifugation was performed at 10,000×g for 10 minutes to separate the expressed recombinant thermophilic arabinose isomerase. The separated recombinant enzyme solution was purified by anion-exchange chromatography (HiPrep Q 16/10, Amersham Bioscience, U.S.A.), ultrafiltration (Centriprep 30, Amicon, Germany) and size-exclusion chromatography; Superdex 200 pg, Amersham Bioscience, U.S.A.) (Table 4). The protein composition of the enzyme solution of each step was analyzed by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) (FIG. 3).

TABLE 4

Purification stage	Total protein (mg)	Total activity (AU)	Purification (%)	Yield (%)
Crude enzyme solution	665	410.4	1.0	100
Heat treatment	480	364.3	1.2	89
Ion exchange chromatography	63.5	201.5	5.1	49
Gel chromatography	28.1	136.3	7.7	33

[0041] The separated and purified recombinant protein was finally identified by N-terminal amino acid sequencing. The protein purified by gel chromatography was analyzed by SDS-PAGE and as a result, it was confirmed that the protein had a molecular weight of approximately 55 kDa, which was the same molecular weight as the *Thermotoga neapolitana*

sp. arabinose isomerase. The protein band was transferred to a PVDF membrane for further investigation by the Korean basic Science Institute. As a result, the 10 amino acids of the amino-terminal of the separated recombinant protein were identified as 'Met-Ile-Leu-Lys-Gln-Tyr-Glu-Phe-Trp' which was the sequence of the N-terminal protein sequence of *Thermotoga neapolitana* sp. arabinose isomerase. Therefore, this sequence was confirmed to be the same as the amino acid sequence of the arabinose isomerase originated from *Thermotoga neapolitana* sp. (SEQ. ID. NO: 3).

Example 5

Reaction Stability of the Recombinant Strain for Enzyme Expression at High Temperature and High Concentration

[0042] *Corynebacterium* is a highly stable strain under various production conditions and has a comparatively hard cell membrane, therefore it remains stable even under high osmotic conditions caused by a high concentration of sucrose. These characteristics of the strain favor the enhancement of reaction stability in high concentration of a substrate. Therefore taking advantage of such characteristics, the present inventors expressed hyperthermophilic arabinose isomerase in the *Corynebacterium*.

[0043] The recombinant *Corynebacterium* strain cultured in a medium prepared in Example 3 and the recombinant *E. coli* strain cultured in LB medium (5 g/L Yeast extract, 10 g/L bacto-trypton, 10 g/L Sodium chloride) were centrifuged respectively at 8000×g for 10 minutes to obtain cells. The cells were resuspended in PBS (phosphate buffered saline) for further experiments. To compare the strain stability at high substrate concentration, 0, 10, 20, 30 and 40% galactose were added to the solution, followed by reaction with stirring at 70° C. for 0, 1, and 2 hours. To quantify the protein expressed in the cells of each reaction product, centrifugation was performed at 13,000×g for 10 minutes and the supernatant was obtained. Then, the expressed proteins were quantified by BCA (bicinchonic acid protein assay method). From the comparison of the protein expression according to the substrate concentration between the recombinant *E. coli* cells and the recombinant *Corynebacterium* cells, it was confirmed that the level of protein expression in *Corynebacterium* was significantly lower, compared with that in the control *E. coli*. In the recombinant *E. coli*, the concentration of galactose was significantly increased as the reaction went on, whereas the expression of the protein in *Corynebacterium* increased approximately 20% even after starting with the highest galactose concentration of this experiment which was 40% (FIG. 4).

[0044] The above results indicate that the protein elution is remarkably reduced in the cells under a high substrate concentration, compared to that in *E. coli*. That is, unlike the conventional *E. coli* host, a *Corynebacterium* host containing

the enzyme expressed therein exhibits reduced elution of intracellular protein under high sugar content.

[0045] *Corynebacterium* host was also confirmed to be very stable under tough reaction conditions including high temperature and high substrate concentration, compared with *E. coli* host. The stability favors the increase of reaction stability in a cell immobilized reactor and thus results in the increase of the half-life of the cell immobilized reactor.

INDUSTRIAL APPLICABILITY

[0046] As explained hereinabove, the stable expression of arabinose isomerase originating from hyperthermophile *Thermotoga* sp. was observed in *Corynebacterium*, and thus immobilized continuous reaction was efficiently carried out in the preferred embodiment of the present invention by using

the *Corynebacterium* containing the active form of the enzyme. To produce biotechnical food material using a microorganism enzyme, an additive must meet a GRAS food grade. The arabinose isomerase originating from the food grade *Thermotoga* sp. prepared by the method of the present invention can be applied to a continuous reaction stably to increase the possibility of industrialization of the enzyme.

[0047] Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the present invention as set forth in the appended claims.

SEQUENCE LISTING

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<220> FEATURE:
<223> OTHER INFORMATION: primer 1

<400> SEQUENCE: 1

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<210> SEQ ID NO 2
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer 2

<400> SEQUENCE: 2

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<210> SEQ ID NO 3
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20 25 30

Arg Ile Val Glu Ala Leu Asn Asn Asp Pro Ile Phe Pro Ser Lys Ile
35 40 45

Val Leu Lys Pro Val Leu Lys Asn Ser Ala Glu Ile Arg Glu Ile Phe
50 55 60

Glu Lys Ala Asn Ala Glu Pro Lys Cys Ala Gly Val Ile Val Trp Met
65 70 75 80

His Thr Phe Ser Pro Ser Lys Met Trp Ile Arg Gly Leu Ser Ile Asn
85 90 95

Lys Lys Pro Leu Leu His Leu His Thr Gln Tyr Asn Arg Glu Ile Pro
100 105 110

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145	150	155 160
Ala Lys Trp Met Arg	Val Ala Cys Ala Ile	Gln Asp Gly Arg Thr Gly
165	170	175
Gln Ile Val Arg Phe	Gly Asp Asn Met Arg	Glu Val Ala Ser Thr Glu
180	185	190
Asp Asp Lys Val Glu	Ala Gln Ile Lys Leu	Gly Trp Ser Ile Asn Thr
195	200	205
Trp Gly Val Gly Glu	Leu Ala Glu Gly Val	Lys Ala Val Pro Glu Asn
210	215	220
Glu Val Glu Glu Leu	Leu Lys Glu Tyr Lys	Glu Arg Tyr Ile Met Pro
225	230	235 240
Glu Asp Glu Tyr Ser	Leu Lys Ala Ile Arg	Glu Gln Ala Lys Met Glu
245	250	255
Ile Ala Leu Arg Glu	Phe Leu Lys Glu Lys	Asn Ala Ile Ala Phe Thr
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Thr Thr Phe Glu Asp	Leu His Asp Leu Pro	Gln Leu Pro Gly Leu Ala
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Val Gln Arg Leu Met	Glu Glu Gly Tyr Gly	Phe Gly Ala Glu Gly Asp
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Pro Gly Asn Glu Leu	Val Leu Gly Ala His	Met Leu Glu Val Cys Pro
340	345	350
Thr Ile Ala Lys Glu	Lys Pro Arg Ile Glu	Val His Pro Leu Ser Ile
355	360	365
Gly Gly Lys Ala Asp	Pro Ala Arg Leu Val	Phe Asp Gly Gln Glu Gly
370	375	380
Pro Ala Val Asn Ala	Ser Ile Val Asp Met	Gly Asn Arg Phe Arg Leu
385	390	395 400
Val Val Asn Arg Val	Leu Ser Val Pro Ile	Glu Arg Lys Met Pro Lys
405	410	415
Leu Pro Thr Ala Arg	Val Leu Trp Lys Pro	Leu Pro Asp Phe Lys Arg
420	425	430
Ala Thr Thr Ala Trp	Ile Leu Ala Gly Gly	Ser His His Thr Ala Phe
435	440	445
Ser Thr Ala Val Asp	Val Glu Tyr Leu Ile	Asp Trp Ala Glu Ala Leu
450	455	460
Glu Ile Glu Tyr Leu	Val Ile Asp Glu Asn	Leu Asp Leu Glu Asn Phe
465	470	475 480
Lys Lys Glu Leu Arg	Trp Asn Glu Leu Tyr	Trp Gly Leu Leu Lys Arg
485	490	495

1. An arabinose isomerase expressed in the *Corynebacterium* sp. strain.

2. The arabinose isomerase according to claim 1, wherein the arabinose isomerase originates from the *Thermotoga* sp., *Thermus* sp. or *Sulforobus* sp. strain having an optimum reaction temperature of 70~90° C.

3. The arabinose isomerase according to claim 2, wherein the arabinose isomerase originates from *Thermotoga neapolitana* DSM 5068.

4. The arabinose isomerase according to claim 1, wherein the arabinose isomerase contains the amino acid sequence represented by SEQ. ID. NO: 3.

5. A method of preparing tagatose from galactose by using the arabinose isomerase of claim 1.

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