

DESCRIPTION

PROCESS FOR PRODUCING SULFUR-CONTAINING α -AMINO ACID
COMPOUND

5

TECHNICAL FIELD

The present invention relates to a process for producing a sulfur-containing α -amino acid compound.

10 BACKGROUND ART

Hitherto, methionine, which is one of sulfur-containing α -amino acid compounds, has been used as an animal feed additive. In a process for producing methionine, acrolein and methyl mercaptan are reacted with each other to produce 3-methylthiopropionaldehyde, and then the 3-methylthiopropionaldehyde obtained is reacted with hydrogen cyanide, ammonia and carbon dioxide to produce 5-(2-methyl-mercaptoethyl)-hydantoin (that is, methionine hydantoin). The resultant product is hydrolyzed under an alkaline condition to give alkali metal methionate, followed by neutralization with an acid such as sulfuric acid or carbonic acid, to liberate methionine (see, for example, JP 55-102557 A).

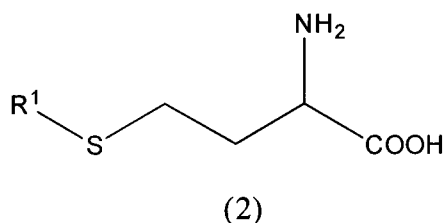
25 DISCLOSURE OF INVENTION

The above-mentioned process employs as C1- or C3- building block hydrogen cyanide and acrolein which require careful safety control in handling and an equipment adopted to such control. Accordingly, there has been demand for a novel process for producing a sulfur-containing α -amino acid compound such as methionine.

An object of the present invention is to provide a novel process for producing sulfur-containing α -amino acid compound such as methionine.

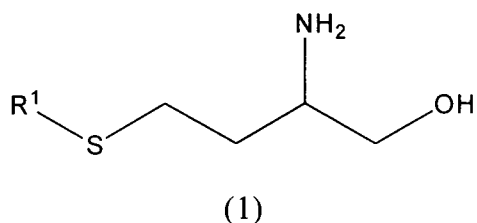
The present invention provides:

[1] A process for producing a sulfur-containing α -amino acid compound represented by the formula (2):



wherein R^1 represents hydrogen, an alkyl group having 1 to 8 carbon atoms, or an aryl group having 6 to 20 carbon atoms;

comprising a step of reacting a sulfur-containing amino alcohol compound represented by the formula (1):



wherein R¹ is the same as defined above;

with a microbial cell or a processed product of the microbial cell of a microorganism capable of converting the sulfur-containing amino alcohol compound into a
5 corresponding sulfur-containing α -amino acid compound (hereinafter, sometimes referred to as "the process of the present invention");

[2] The process according to the item [1] wherein the microorganism is capable of preferentially oxidizing the
10 hydroxyl group of the sulfur-containing amino alcohol compound;

[3] The process according to the item [1] wherein the microorganism is one or more microorganisms selected from a group consisting of microorganisms of the genus *Alcaligenes*,
15 microorganisms of the genus *Bacillus*, microorganisms of the genus *Pseudomonas*, microorganisms of the genus *Rhodobacter* and microorganisms of the genus *Rhodococcus*;

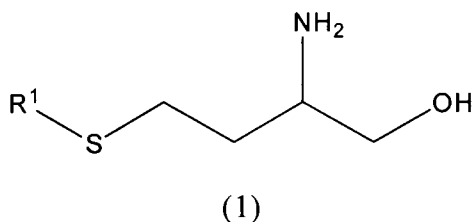
[4] The process according to the item [1] wherein the microorganism is one or more microorganisms selected from a
20 group consisting of *Alcaligenes denitrificans*, *Alcaligenes eutrophus*, *Alcaligenes faecalis*, *Alcaligenes* sp., *Alcaligenes xylosoxydans*, *Bacillus alvei*, *Bacillus badius*, *Bacillus brevis*, *Bacillus cereus*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus licheniformis*, *Bacillus moritai*,
25 *Bacillus pumilus*, *Bacillus sphaericus*, *Bacillus subtilis*,

Bacillus validus, *Pseudomonas denitrificans*, *Pseudomonas*
ficuserectae, *Pseudomonas fragi*, *Pseudomonas mendocina*,
Pseudomonas oleovorans, *Pseudomonas ovalis*, *Pseudomonas*
pseudoalcaligenes, *Pseudomonas putida*, *Pseudomonas*
5 *putrefaciens*, *Pseudomonas riboflavina*, *Pseudomonas*
straminea, *Pseudomonas syringae*, *Pseudomonas tabaci*,
Pseudomonas taetrolens, *Pseudomonas vesicularis*,
Rhodobacter sphaeroides, *Rhodococcus erythropolis*,
Rhodococcus groberulus, *Rhodococcus rhodochrous* and
10 *Rhodococcus* sp.;

[5] The process according to any one of the items [1] to
[4] wherein R^1 of the sulfur-containing amino alcohol
compound is an alkyl group having 1 to 8 carbon atoms;

[6] The process according to any one of the items [1] to
15 [5] wherein R^1 of the sulfur-containing amino alcohol
compound is a methyl group; and

[7] Use of a microbial cell or a processed product of the
microbial cell of a microorganism as a catalyst for
converting a sulfur-containing amino alcohol compound
20 represented by the formula (1):



wherein R^1 represents hydrogen, an alkyl group having 1 to

8 carbon atoms, or an aryl group having 6 to 20 carbon atoms;

into the corresponding sulfur-containing α -amino acid compound, wherein the microorganism is capable of preferentially oxidizing the hydroxyl group of the sulfur-containing amino alcohol compound (hereinafter, sometimes referred to as "the use of the present invention").

The present invention is capable of providing a novel process for producing a sulfur-containing α -amino acid compound such as methionine.

MODE FOR CARRYING OUT THE INVENTION

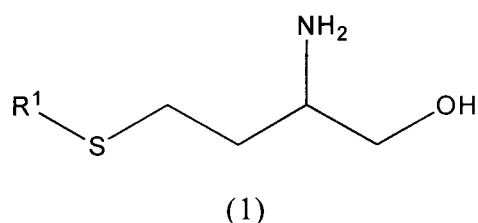
It is considered that the inventions described herein is not limited to the particular methodologies, protocols, and reagents described herein and that they can be modified. It is considered that the terms used herein are meant only to describe a particular embodiment of the present invention, and that such terms do not limit the scope of the present invention.

Unless otherwise noted, all of the technical terms and chemical terms used herein have the same meaning as those commonly understood by a person skilled in the technical field of the present invention. While the present invention may be carried out or examined by using methods or materials similar or equivalent to those described

herein, some of the preferred methods, equipments, and materials are described in the following.

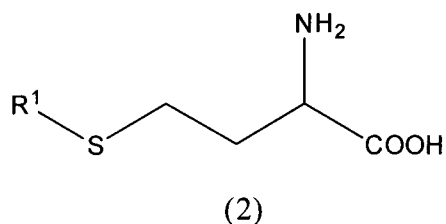
Hereinafter, the present invention is explained in more detail.

5 The process of the present invention comprises a step of reacting a sulfur-containing amino alcohol compound represented by the formula (1):



10 wherein R¹ represents hydrogen, an alkyl group having 1 to 8 carbon atoms, or an aryl group having 6 to 20 carbon atoms [hereinafter, sometimes referred to as "Compound (1)"];

15 with a microbial cell or a processed product of the microbial cell of a microorganism capable of converting the sulfur-containing amino alcohol compound into a corresponding sulfur-containing α-amino acid compound, i.e. a compound represented by the formula (2):



wherein R¹ is the same as defined above [hereinafter,

sometimes referred to as "Compound (2)"].

Examples of "an alkyl group having 1 to 8 carbon atoms" represented by R^1 in Compound (1) and Compound (2) include a methyl group, an ethyl group, a propyl group, an isopropyl group, a butyl group, an isobutyl group, a t-butyl group, a pentyl group, a hexyl group, a heptyl group, and an octyl group. Examples of "an aryl group having 6 to 20 carbon atoms" represented by R^1 include a phenyl group, a tolyl group, and a naphthyl group.

Preferred examples of R^1 include an alkyl group having 1 to 8 carbon atoms. More preferred examples of R^1 include a methyl group.

The microbial cell or the processed product of the microbial cell of a microorganism capable of preferentially oxidizing the hydroxyl group of the sulfur-containing amino alcohol compound, as a catalyst to be used in the process of the present invention, has an ability to convert Compound (1) into Compound (2). The term "preferentially oxidize" used herein means that the oxidation of a hydroxyl group proceeds preferentially to the oxidation of a sulfide group in the sulfur-containing amino alcohol compound.

Examples of the microorganism having the above ability (hereinafter, sometimes referred to as "the present microorganism") include one or more microorganisms selected

from a group consisting of microorganisms of the genus *Alcaligenes*, microorganisms of the genus *Bacillus*, microorganisms of the genus *Pseudomonas*, microorganisms of the genus *Rhodobacter* and microorganisms of the genus *Rhodococcus*.

Examples of the microorganism having the above ability (i.e. the present microorganisms) also include one or more microorganisms selected from a group consisting of *Alcaligenes denitrificans*, *Alcaligenes eutrophus*, *Alcaligenes faecalis*, *Alcaligenes* sp., *Alcaligenes xylosoxydans*, *Bacillus alvei*, *Bacillus badius*, *Bacillus brevis*, *Bacillus cereus*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus licheniformis*, *Bacillus moritai*, *Bacillus pumilus*, *Bacillus sphaericus*, *Bacillus subtilis*, *Bacillus validus*, *Pseudomonas denitrificans*, *Pseudomonas ficuserectae*, *Pseudomonas fragi*, *Pseudomonas mendocina*, *Pseudomonas oleovorans*, *Pseudomonas ovalis*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas putida*, *Pseudomonas putrefaciens*, *Pseudomonas riboflavina*, *Pseudomonas straminea*, *Pseudomonas syringae*, *Pseudomonas tabaci*, *Pseudomonas taetrolens*, *Pseudomonas vesicularis*, *Rhodobacter sphaeroides*, *Rhodococcus erythropolis*, *Rhodococcus groberulus*, *Rhodococcus rhodochrous* and *Rhodococcus* sp..

Preferred examples of the microorganism having the

above ability include one or more microorganisms selected from a group consisting of *Alcaligenes denitrificans* JCM5490, *Alcaligenes eutrophus* ATCC43123, *Alcaligenes faecalis* IF012669, *Alcaligenes* sp. IF014130, *Alcaligenes* xylosoxydans IF015125t, *Alcaligenes xylosoxydans* IF015126t, *Bacillus alvei* IF03343t, *Bacillus badius* ATCC14574t, *Bacillus brevis* JCM2503t, *Bacillus cereus* JCM2152t, *Bacillus coagulans* JCM2257t, *Bacillus firmus* JCM2512t, *Bacillus licheniformis* ATCC27811, *Bacillus licheniformis* IF012197, *Bacillus licheniformis* IF012200t, *Bacillus moritai* ATCC21282, *Bacillus pumilus* IF012092t, *Bacillus sphaericus* IF03341, *Bacillus sphaericus* IF03526, *Bacillus subtilis* ATCC14593, *Bacillus subtilis* ATCC15841, *Bacillus subtilis* IF03108, *Bacillus subtilis* IF03132, *Bacillus subtilis* IF03026, *Bacillus subtilis* IF03037, *Bacillus subtilis* IF03108, *Bacillus subtilis* IF03134, *Bacillus validus* IF013635, *Pseudomonas denitrificans* IAM1426, *Pseudomonas denitrificans* IAM1923, *Pseudomonas ficuserectae* JCM2400t, *Pseudomonas fragi* IAM12402, *Pseudomonas fragi* IF03458t, *Pseudomonas mendocina* IF014162, *Pseudomonas oleovorans* IF013583t, *Pseudomonas ovalis* IF012688, *Pseudomonas pseudoalcaligenes* JCM5968t, *Pseudomonas putida* IF012996, *Pseudomonas putida* IF014164t, *Pseudomonas putida* IF03738, *Pseudomonas putida* IF012653, *Pseudomonas putrefaciens* IF03910, *Pseudomonas riboflavina* IF013584t,

Pseudomonas straminea JCM2783t, *Pseudomonas syringae* IFO14055, *Pseudomonas tabaci* IFO3508, *Pseudomonas taetrolens* IFO3460, *Pseudomonas vesicularis* JCM1477t, *Rhodobacter sphaeroides* ATCC17023, *Rhodococcus erythropolis* IFO12320, *Rhodococcus groberulus* ATCC15076, *Rhodococcus rhodochrous* ATCC15076, *Rhodococcus rhodochrous* ATCC15610, *Rhodococcus rhodochrous* ATCC19067, *Rhodococcus rhodochrous* ATCC19149, *Rhodococcus rhodochrous* ATCC19150, *Rhodococcus rhodochrous* ATCC21197, *Rhodococcus rhodochrous* ATCC21199, *Rhodococcus rhodochrous* JCM3202t, *Rhodococcus* sp. ATCC19070, *Rhodococcus* sp. ATCC19071 and *Rhodococcus* sp. ATCC19148.

These microorganisms may be either isolated from natural sources, or easily gotten by purchasing from culture collection.

Examples of culture collections from which the microorganisms can be purchased, include the following culture collections.

1. IFO (Institute of Fermentation Osaka) culture collection

At present, the culture collection is transferred to National Institute of Technology and Evaluation Biological Resource Center (NBRC). Microorganisms can be purchased by filing an application to NBRC, which can be done by, for example, accessing the website of NBRC (<http://www.nbrc.nite.go.jp/NBRC2/NBRCDispSearchServlet?lang=jp>).

2. ATCC (American Type Culture Collection)

Microorganisms can be purchased through Summit Pharmaceuticals International Corporation, ATCC Industry Division by, for example, accessing its website
5 (http://www.summitpharma.co.jp/japanese/service/s_ATCC.html). Alternatively, microorganisms can be purchased directly from ATCC.

3. JCM (Japan Collection of Microorganisms)

At present, the culture collection is transferred to
10 National Institute of Physical and Chemical Research Biological Resource Center (RIKEN BRC), Microbe Division. Microorganisms can be purchased by filing an application to RIKEN BRC, which can be done by, for example, accessing a site for the culture collection in the website of RIKEN
15 (http://www.jcm.riken.go.jp/JCM/aboutJCM_J.shtml).

4. IAM Culture Collection

At present, among the IAM Culture Collection, bacteria, yeasts, and filamentous fungi are transferred to National Institute of Physical and Chemical Research Biological
20 Resource Center, Microbe Division (JCM), and microalgae are transferred to Microbial Culture Collection in National Institute for Environmental Studies (NIES). Microorganisms can be purchased by filing an application to JCM or NIES, which can be done by, for example, accessing a site for the
25 culture collections in the website of JCM

(http://www.jcm.riken.go.jp/JCM/aboutJCM_J.shtml) or in the website of NIES (<http://mcc.nies.go.jp/aboutOnlineOrder.do>).

The microbial cell or the processed products of the microbial cell of a microorganism capable of preferentially oxidizing the hydroxyl group of the sulfur-containing amino alcohol compound, as a catalyst to be used in the process of the present invention, may also be obtained and prepared by screening microorganisms capable of converting Compound (1) into Compound (2). Specifically, for example, in a test tube is placed 5 ml of sterilized culture medium, and thereto is inoculated with a microorganism obtained by purchasing from the above-mentioned culture collection or a microorganism isolated from soils. The resultant is incubated with shaking at 30 °C under an aerobic condition. After the completion of the incubation, microbial cells are collected by centrifugation to obtain viable cells. In a screw-top test tube is placed 2 ml of 0.1 M Tris-glycine buffer (pH 10), and thereto are added the above-prepared viable cells, and the mixture is suspended. To the suspension is added 2 mg of methioninol, and the resultant mixture is shaken at 30 °C for 3 to 7 days. After the completion of the reaction, 1 ml of the reaction solution is sampled. The cells are removed from the sampling solution, and the amount of the produced methionine is analyzed by liquid chromatography.

Thus, microorganisms capable of preferentially oxidizing the hydroxyl group of the sulfur-containing amino alcohol compound are screened.

5 Hereinafter, a method for the preparation of the present microorganisms is explained.

The present microorganisms may be cultured in a culture medium for culturing various microorganisms which appropriately contain a carbon source, a nitrogen source,
10 an organic salt, an inorganic salt, and so on.

Examples of the carbon source include sugars such as glucose, dextrin and sucrose; sugar alcohols such as glycerol; organic acids such as fumaric acid, citric acid and pyruvic acid; animal oils; vegetable oils; and molasses.
15 These carbon sources are added to the culture medium in an amount of usually about 0.1 % (w/v) to 30 % (w/v) of the culture.

Examples of the nitrogen source include natural organic nitrogen sources such as meat extract, peptone,
20 yeast extract, malt extract, soy flour, Corn Steep Liquor, cottonseed flour, dried yeast and casamino acids; amino acids; sodium salts of inorganic acids such as sodium nitrate; ammonium salts of inorganic acids such as ammonium chloride, ammonium sulfate and ammonium phosphate; ammonium
25 salts of organic acids such as ammonium fumarate and

ammonium citrate; and urea. Among these nitrogen sources, ammonium salts of organic acids, natural organic nitrogen sources, and amino acids and others may also be used as carbon sources in many cases. The above nitrogen sources
5 are added to the culture medium in an amount of usually about 0.1 % (w/v) to 30 % (w/v) of the culture.

Examples of the organic salt and inorganic salt include chloride, sulfate, acetate, carbonate and phosphate of potassium, sodium, magnesium, iron, manganese, cobalt,
10 zinc, and others. Specific examples thereof include sodium chloride, potassium chloride, magnesium sulfate, ferrous sulfate, manganese sulfate, cobalt chloride, zinc sulfate, copper sulfate, sodium acetate, calcium carbonate, potassium hydrogen phosphate and dipotassium hydrogen
15 phosphate. These organic salts and/or inorganic salts are added to the culture medium in an amount of usually about 0.0001 % (w/v) to 5 % (w/v) of the culture.

Examples of the culture method include solid culture and liquid culture (e.g. a test tube culture, a flask
20 culture, or a jar fermenter culture).

Culture temperature and pH of the culture are not particularly limited as long as the present microorganisms are able to grow in the range thereof. For example, the culture temperature may be in a range of about 15 °C to
25 about 45 °C, and the pH of the culture may be in a range of

about 4 to about 8. The culture time may be appropriately selected depending on the culture conditions, and is usually about 1 day to about 7 days.

The microbial cells of the present microorganisms can be directly used as a catalyst for the process of the present invention. Among methods for using the microbial cells of the present microorganisms, examples of a method for directly using the microbial cells of the present microorganisms include:

- (1) a method for directly using culture, and
- (2) a method for using microbial cells collected by centrifuging a culture (wet microbial cells washed as needed with buffer or water).

The processed products of the microbial cells of the present microorganisms may also be used as a catalyst for the process of the present invention. Examples of the processed products of the microbial cells include microbial cells obtained by culturing followed by treating with an organic solvent (e.g. acetone and ethanol), lyophilizing, or treating with alkali; physically or enzymatically disrupted microbial cells; and crude enzymes separated or extracted from the these microbial cells. Furthermore, examples of the processed products include those immobilized by a known method after the above-mentioned treatments.

Specific embodiments include the microbial cells of the present microorganisms and the processed products thereof (e.g. cell-free extracts, partially purified proteins, purified proteins and immobilized materials thereof). Examples of the processed products of the microbial cells include lyophilized microorganisms, organic solvent-treated microorganisms, dried microorganisms, disrupted microorganisms, autolysates of microorganisms, sonicated microorganisms, extracts of microorganisms, and alkali-treated microorganisms. Examples of a method of obtaining the immobilized materials include carrier binding methods (e.g. a method of adsorbing proteins and others onto inorganic carriers such as silica gel and ceramics, cellulose, or ion-exchanged resin) and encapsulating methods [e.g. a method of trapping proteins and others in a network structure of macromolecules such as polyacrylamide, sulfur-containing polysaccharide gel (e.g. carrageenan gel), alginate gel, and agar gel].

In the event that the present microorganisms are used in the industrial production process, the products of killed microorganisms might be preferred to unprocessed microorganisms from the point of view of limitation of manufacturing equipments or other factors. Examples of a method for killing the microorganisms include physical sterilization (e.g. heating, drying, freezing, irradiation,

sonication, filtration, and electric sterilization) and sterilization with chemical agents (e.g. alkalis, acids, halogens, oxidizing agents, sulfur, boron, arsenic, metals, alcohols, phenols, amines, sulfides, ethers, aldehydes, ketones, cyan, and antibiotics). Among these killing methods, generally, it is preferable to select a method which can lower the amount of residues or contaminants in the reaction system and can minimize inactivation of the above-described ability of the present microorganism to preferentially oxidize the hydroxyl group of the sulfur-containing amino alcohol compound.

The process of the present invention is usually carried out in the presence of water. The water used in this case may be in the form of a buffer. Examples of buffering agents used in the buffer include alkali metal salts of phosphoric acid such as sodium phosphate and potassium phosphate, and alkali metal salts of acetic acid such as sodium acetate and potassium acetate. Examples of alkaline buffer include Tris-HCl buffer and Tris-citrate buffer.

The process of the present invention may also be carried out by additionally using a hydrophobic organic solvent, i.e. in the presence of water and the hydrophobic organic solvent. Examples of the hydrophobic organic

solvent used in this case include esters such as ethyl formate, ethyl acetate, propyl acetate, butyl acetate, ethyl propionate and butyl propionate, alcohols such as n-butyl alcohol, n-amyl alcohol and n-octyl alcohol, aromatic hydrocarbons such as benzene, toluene and xylene, ethers such as diethylether, diisopropylether and methyl-t-butylether, halogenated hydrocarbons such as chloroform and 1,2-dichloroethane, and mixtures thereof.

The process of the present invention may also be carried out by additionally using a hydrophilic organic solvent, i.e. in the presence of water and an aqueous medium. Examples of the hydrophilic organic solvent used in this case include alcohols such as methanol and ethanol, ketones such as acetone, ethers such as dimethoxyethane, tetrahydrofuran and dioxane, dimethylsulfoxide, and mixtures thereof.

While the process of the present invention is usually carried out in a range of pH of aqueous layer of 3 to 11, the pH may be appropriately changed in such a range that the reaction proceeds. It is preferable that the process of the present invention be carried out in the alkaline range, and it is more preferable that the process be carried out in a range of pH of aqueous layer of 8 to 10.

While the process of the present invention is usually carried out in a range of about 0 °C to about 60 °C, the

temperature may be appropriately changed in such a range that the reaction proceeds.

The process of the present invention is usually carried out in a range of for about 0.5 hours to about 10 days. After the completion of adding the sulfur-containing amino alcohol compound represented by the formula (1) [i.e. Compound (1)], which is the starting compound, the endpoint of the reaction can be checked, for example, by measuring the amount of the sulfur-containing amino alcohol compound of the formula (1) in the reaction solution by liquid chromatography or gas chromatography and the others.

The concentration of the sulfur-containing amino alcohol compound represented by the formula (1) [i.e. Compound (1)], which is the starting compound used in the process of the present invention, is usually 50 % (w/v) or less and the sulfur-containing amino alcohol compound of the formula (1) [i.e. Compound (1)] may be continuously or successively added to a reaction system in order to maintain the concentration of the sulfur-containing amino alcohol compound of the formula (1) in the reaction system nearly constant.

During the process of the present invention, for example, a sugar such as glucose, sucrose or fructose, or a surfactant such as Triton X-100 (registered trade mark) or Tween 60 (registered trade mark) may be added to the

reaction system if necessary.

The recover of the sulfur-containing α -amino acid compound represented by the formula (2) from the reaction solution may be carried out by any methods known in the art.

5 Example of the method include purification by performing post-treatment of the reaction solution such as organic solvent extraction, concentration, ion exchange method and crystallization, if necessary in combination with column chromatography, and distillation and others.

10 The sulfur-containing amino acid compound represented by the formula (2) prepared by the process of the present invention may be in the form of a salt.

EXAMPLES

15 Hereinafter, the present invention is explained in more detail with some examples, which should not be construed to be limited thereto.

Example 1

20 Production of the sulfur-containing α -amino acid compound from the sulfur-containing amino alcohol compound according to the process of the present invention

25 In a test tube was placed 5 ml of sterilized culture medium, which was prepared by adding polypeptone (5 g), yeast extract (3 g), meat extract (3 g), ammonium sulfate (0.2 g), potassium dihydrogen phosphate (1 g) and magnesium

sulfate heptahydrate (0.5 g) to 1 L of water and adjusting the pH to 7.0, and thereto was inoculated with each cells of the microorganisms shown in Table 1. The resultant was incubated with shaking at 30 °C under an aerobic condition.

5 After the completion of the incubation, the microbial cells were collected by centrifugation to obtain viable cells. In a screw-top test tube was placed 2 ml of 0.1 M Tris-glycine buffer (pH 10), and thereto was added the above-prepared viable cells, and the mixture was suspended. To
10 the suspension was added 2 mg of methioninol, and the resultant mixture was shaken at 30 °C for 3 to 7 days.

After the completion of the reaction, 1 ml of the reaction solution was sampled. The cells were removed from the sampling solution, and the amount of the produced
15 methionine was analyzed by liquid chromatography. The results are shown in Table 1.

Conditions for content analysis

Column: Cadenza CD-C18 (4.6 mmφ × 15 cm, 3 μm)
20 (manufactured by Imtakt Corp.)

Mobile phase: 0.1 % aqueous trifluoroacetic acid as Solution A, and methanol as Solution B

Time (minutes)	Solution A (%) : Solution B (%)
0	100 : 0
25 10	100 : 0

20 50 : 50

25 50 : 50

25.1 100 : 20

Flow rate: 0.5 ml/min.

5 Column temperature: 40 °C

Detection: 220 nm

Table 1

Name of strain	Methionine yield (%)
<i>Alcaligenes denitrificans</i> JCM 5490	15.9
<i>Alcaligenes eutrophus</i> ATCC 43123	18.2
<i>Alcaligenes faecalis</i> IFO 12669	13.7
<i>Alcaligenes</i> sp. IFO 14130	16.5
<i>Alcaligenes xylosoxydans</i> IFO15125t <i>denitrificans</i>	16.8
<i>Alcaligenes xylosoxydans</i> IFO15126t <i>xylosoxydans</i>	15.8
<i>Bacillus alvei</i> IFO 3343t	14.3
<i>Bacillus badius</i> ATCC 14574t	15.6
<i>Bacillus brevis</i> JCM 2503t	15.0
<i>Bacillus cereus</i> JCM 2152t	17.0
<i>Bacillus coagulans</i> JCM 2257t	10.7
<i>Bacillus firmus</i> JCM 2512t	18.3
<i>Bacillus licheniformis</i> ATCC 27811	16.6
<i>Bacillus licheniformis</i> IFO 12197	17.0
<i>Bacillus licheniformis</i> IFO 12200t	10.5
<i>Bacillus moritai</i> ATCC 21282	16.7
<i>Bacillus pumilus</i> IFO 12092t	16.2
<i>Bacillus sphaericus</i> IFO 3341	17.2
<i>Bacillus sphaericus</i> IFO 3526	18.3
<i>Bacillus subtilis</i> ATCC 14593	13.9
<i>Bacillus subtilis</i> ATCC15841	12.1
<i>Bacillus subtilis</i> IFO 03108	14.0
<i>Bacillus subtilis</i> IFO 03134	15.0
<i>Bacillus subtilis</i> IFO 3026	18.9
<i>Bacillus subtilis</i> IFO 3037	14.3
<i>Bacillus subtilis</i> IFO 3108	13.3
<i>Bacillus subtilis</i> IFO 3134	12.6
<i>Bacillus validus</i> IFO 13635	14.4
<i>Pseudomonas denitrificans</i> IAM 1426	17.2
<i>Pseudomonas denitrificans</i> IAM 1923	36.5
<i>Pseudomonas ficuserectae</i> JCM 2400t	19.3
<i>Pseudomonas fragi</i> IAM 12402	16.0

<i>Pseudomonas fragi</i> IFO 3458t	34.3
<i>Pseudomonas mendocina</i> IFO 14162	26.8
<i>Pseudomonas oleovorans</i> IFO 13583t	19.6
<i>Pseudomonas ovalis</i> IFO 12688	12.7
<i>Pseudomonas pseudoalcaligenes</i> JCM 5968t	15.2
<i>Pseudomonas putida</i> IFO 12996	14.3
<i>Pseudomonas putida</i> IFO 14164t	14.4
<i>Pseudomonas putida</i> IFO 3738	29.2
<i>Pseudomonas putida</i> IFO12653	12.8
<i>Pseudomonas putrefaciens</i> IFO 3910	16.4
<i>Pseudomonas riboflavina</i> IFO 13584t	11.9
<i>Pseudomonas straminea</i> JCM 2783t	23.7
<i>Pseudomonas syringae</i> subsp. <i>syringae</i> IFO14055	23.7
<i>Pseudomonas tabaci</i> IFO 3508	12.5
<i>Pseudomonas taetrolens</i> IFO 3460	18.1
<i>Pseudomonas vesicularis</i> JCM 1477t	15.3
<i>Rhodobacter sphaeroides</i> ATCC 17023	15.7
<i>Rhodococcus erythropolis</i> IFO 12320	12.4
<i>Rhodococcus globerulus</i> ATCC 15076	28.9
<i>Rhodococcus rhodochrous</i> ATCC 15610	22.3
<i>Rhodococcus rhodochrous</i> ATCC 19067	25.4
<i>Rhodococcus rhodochrous</i> ATCC 19149	22.5
<i>Rhodococcus rhodochrous</i> ATCC 19150	24.9
<i>Rhodococcus rhodochrous</i> ATCC 21197	26.5
<i>Rhodococcus rhodochrous</i> ATCC 21199	27.2
<i>Rhodococcus rhodochrous</i> JCM 3202t	38.5
<i>Rhodococcus</i> sp ATCC 19070	22.5
<i>Rhodococcus</i> sp ATCC 19071	35.8
<i>Rhodococcus</i> sp ATCC 19148	24.8

Reference example 1

Screening microorganisms capable of converting the sulfur-
5 containing amino alcohol compound into a corresponding
sulfur-containing α -amino acid compound

In a test tube is placed 5 ml of sterilized culture
medium, which is prepared by adding polypeptone (5 g),
yeast extract (3 g), meat extract (3 g), ammonium sulfate
10 (0.2 g), potassium dihydrogen phosphate (1 g) and magnesium
sulfate heptahydrate (0.5 g) to 1 L of water and then

adjusting the pH to 7.0, and thereto is inoculated with a microorganism obtained by purchasing from the previously mentioned culture collection or a microorganism isolated from soils. The resultant is incubated with shaking at 30 °C under an aerobic condition. After the completion of the incubation, microbial cells are collected by centrifugation to obtain viable cells. In a screw-top test tube is placed 2 ml of 0.1 M Tris-glycine buffer (pH 10), and thereto is added the above-prepared viable cells, and the mixture is suspended. To the suspension is added 2 mg of methioninol, and the resultant mixture is shaken at 30 °C for 3 to 7 days.

After the completion of the reaction, 1 ml of the reaction solution is sampled. The cells are removed from the sampling solution, and the amount of the produced methionine is analyzed by liquid chromatography.

Thus, microorganisms capable of converting the sulfur-containing amino alcohol compound into the corresponding sulfur-containing α -amino acid compound are screened.

20

Conditions for content analysis

Column: Cadenza CD-C18 (4.6 mm ϕ × 15 cm, 3 μ m)
(manufactured by Imtakt Corp.)

Mobile phase: 0.1 % aqueous trifluoroacetic acid as

25 Solution A, methanol as Solution B

	Time (minutes)	Solution A (%) : Solution B (%)
	0	100 : 0
	10	100 : 0
	20	50 : 50
5	25	50 : 50
	25.1	100 : 20

Flow rate: 0.5 ml/min

Column temperature: 40 °C

Detection: 220 nm

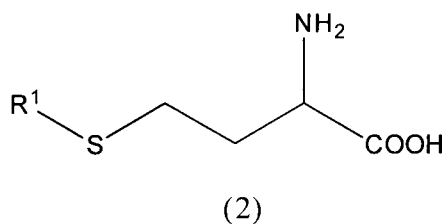
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INDUSTRIAL APPLICABILITY

The present invention can provide a novel process for producing a sulfur-containing α -amino acid compound such as methionine.

CLAIMS

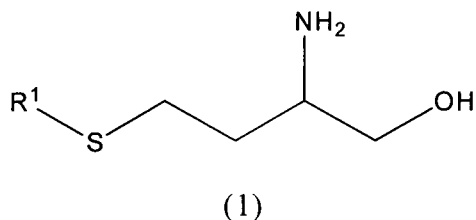
1. A process for producing a sulfur-containing α -amino acid compound represented by the formula (2):



wherein R^1 represents hydrogen, an alkyl group having 1 to 8 carbon atoms, or an aryl group having 6 to 20 carbon atoms;

comprising a step of reacting a sulfur-containing amino alcohol compound represented by the formula (1):

10



wherein R^1 is the same as defined above,
with a microbial cell or a processed product of the microbial cell of a microorganism capable of converting the sulfur-containing amino alcohol compound into a corresponding sulfur-containing α -amino acid compound.

15

2. The process according to claim 1 wherein the microorganism is capable of preferentially oxidizing the hydroxyl group of the sulfur-containing amino alcohol

compound.

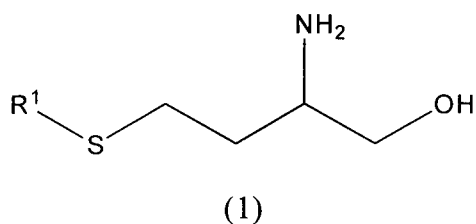
3. The process according to claim 1 wherein the microorganism is one or more microorganisms selected from a group consisting of microorganisms of the genus *Alcaligenes*,
5 microorganisms of the genus *Bacillus*, microorganisms of the genus *Pseudomonas*, microorganisms of the genus *Rhodobacter* and microorganisms of the genus *Rhodococcus*.

4. The process according to claim 1 wherein the
10 microorganism is one or more microorganisms selected from a group consisting of *Alcaligenes denitrificans*, *Alcaligenes eutrophus*, *Alcaligenes faecalis*, *Alcaligenes* sp., *Alcaligenes xylosoxydans*, *Bacillus alvei*, *Bacillus badius*, *Bacillus brevis*, *Bacillus cereus*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus licheniformis*, *Bacillus moritai*,
15 *Bacillus pumilus*, *Bacillus sphaericus*, *Bacillus subtilis*, *Bacillus validus*, *Pseudomonas denitrificans*, *Pseudomonas ficuserectae*, *Pseudomonas fragi*, *Pseudomonas mendocina*, *Pseudomonas oleovorans*, *Pseudomonas ovalis*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas putida*, *Pseudomonas putrefaciens*,
20 *Pseudomonas riboflavina*, *Pseudomonas straminea*, *Pseudomonas syringae*, *Pseudomonas tabaci*, *Pseudomonas taetrolens*, *Pseudomonas vesicularis*, *Rhodobacter sphaeroides*, *Rhodococcus erythropolis*, *Rhodococcus groberulus*, *Rhodococcus rhodochrous* and
25 *Rhodococcus* sp..

5. The process according to any one of claims 1 to 4 wherein R¹ of the sulfur-containing amino alcohol compound is an alkyl group having 1 to 8 carbon atoms.

6. The process according to any one of claims 1 to 5 wherein R¹ of the sulfur-containing amino alcohol compound is a methyl group.

7. Use of a microbial cell or a processed product of the microbial cell of a microorganism as a catalyst for converting a sulfur-containing amino alcohol compound represented by the formula (1):



wherein R¹ represents hydrogen, an alkyl group having 1 to 8 carbon atoms, or an aryl group having 6 to 20 carbon atoms;

into a corresponding sulfur-containing α-amino acid compound, wherein the microorganism is capable of preferentially oxidizing the hydroxyl group of the sulfur-containing amino alcohol compound.