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Production of N^{α}-benzyloxycarbonyl-L-aminoadipic acid and N^{α}-benzyloxycarbonyl-D-aminoadipic acid with *Rhodococcus* sp. AIU Z-35-1

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Abstract

We isolated a new bacterial strain capable of producing N^{α}-benzyloxycarbonyl-L-aminoadipic acid (N^{α}-Z-L-AAA) and N^{α}-Z-D-AAA by cell reaction. This isolated strain was identified as a member of the genus *Rhodoccocus*. By this strain, N^{α}-Z-L-AAA and N^{α}-Z-D-AAA were formed from N^{α}-Z-L-lysine and N^{α}-Z-D-lysine via N^{α}-Z-L-aminoadipate-δ-semialdehyde (N^{α}-Z-L-AASA) and N^{α}-Z-D-AASA, respectively, and a more than 10-fold higher concentration of N^{α}-Z-L-AAA was produced in one-third the reaction time of *Aspergillus niger* AKU 3302. In addition, approximately 40 mM N^{α}-Z-D-AAA was produced from 50 mM N^{α}-Z-D-lysine. Thus, the production of N^{α}-Z-L-AAA and N^{α}-Z-D-AAA was remarkably improved using the new isolated strain.

Keywords: N^{α}-Z-L-lysine; N^{α}-Z-D-lysine; N^{α}-Z-L-aminoadipate-δ-semialdehyde; N^{α}-Z-D-aminoadipate-δ-semialdehyde; N^{α}-Z-L-aminoadipic acid; N^{α}-Z-D-aminoadipic acid

1. Introduction

L-α-Aminoadipic acid (L-α-AAA) is a precursor of β-lactam antibiotics, and L-α-AAA and its related compounds provide interesting raw materials for the chemical synthesis of new antibiotics or physiological peptides. Some enzymatic and microbial methods for L-α-AAA production have recently been developed using ketoaminoadipic acid or L-lysine as starting material [1,2], but these methods had drawbacks such as requiring a cofactor regeneration system or a second substrate. Since no addition of other materials except for enzymes and a substrate is generally desirable for obtaining the pure product, we developed new methods for the production of N^{α}-Z-L-AASA and N^{α}-Z-L-AAA from N^{α}-Z-L-lysine utilizing amine oxidase and mycelia of *Aspergillus niger* AKU 3302, respectively [3,4]. However, the yield of N^{α}-Z-L-AAA was not high, and little N^{α}-Z-D-AAA was formed by the method with mycelia of *A. niger*. In addition, the cultivation of *A. niger* was more difficult than that of bacteria in a liquid medium. Therefore, we further screened for a bacterial strain capable of producing N^{α}-Z-L-AAA and N^{α}-Z-D-AAA with a high conversion yield. We here describe the production of N^{α}-Z-L-AAA and N^{α}-Z-D-AAA by the cell reaction with the isolated strain, together with the screening of bacterial strain for the production of N^{α}-Z-L-AAA and N^{α}-Z-D-AAA, and the identification of the isolated strain.

Abbreviations: MBTH, 3-methyl-2-benzothiazolinone hydrazone; N^{α}-Z-L-lysine, N^{α}-benzyloxycarbonyl-L-lysine

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2. Materials and methods

2.1. Chemicals

N^{α}-Z-L-lysine and N^{α}-Z-D-lysine were purchased from Calbiochem–Novabiochem (Läufelfingen, Switzerland) and Fluka Chemie (Buchs, Switzerland), respectively. N^{α}-Z-L-AAA and N^{α}-Z-D-AAA were obtained from Sanyo Fine (Osaka, Japan). All other chemicals used were of analytical grade and commercially available.

2.2. Isolation of bacterial strains

An enrichment culture was carried out using a N^{α}-Z-L-lysine medium, pH 7.0, consisting of 0.2% KH_2PO_4, 0.1% Na_2HPO_4, 0.05% $MgSO_4{\cdot}7H_2O$, 0.5% glucose, and 0.5% N^{α}-Z-L-lysine. The enrichment broth was transferred to agar plates of the same components as the N^{α}-Z-L-lysine medium, and the plates were then incubated at 30 °C for 5 days. Approximately 200 bacterial strains were isolated at random from the plates, and each isolated strain was inoculated into 5 ml of the N^{α}-Z-L-lysine medium in a test tube (Ø 1.6 cm × 18 cm). After cultivation at 30 °C for 5 days with shaking, the culture supernatant was separated from the cells by centrifugation at 20,000 × *g* for 10 min. The utilization of N^{α}-Z-L-lysine was analyzed with TLC using their culture supernatant, and strains utilizing N^{α}-Z-L-lysine with high speed were selected. The cells of the selected strains were then incubated with 20 mM N^{α}-Z-L-lysine at 30 °C for 4 days. The reaction products from N^{α}-Z-L-lysine were first analyzed with TLC, and the amount of N^{α}-Z-L-AAA formed was then analyzed by HPLC with a TSK-Gel DEAE-5PW column (Tosoh, Tokyo, Japan) as described below. In the strains exhibiting the formation of N^{α}-Z-L-AAA, the formation of N^{α}-Z-D-AAA was also investigated by the cell reaction with N^{α}-Z-D-lysine. The bacterial strain with the highest productivity of N^{α}-Z-L-AAA and N^{α}-Z-D-AAA, strain Z-35-1, was finally selected and used in this study.

2.3. Taxonomic studies of strain Z-35-1

The strain was cultivated on a medium of nutrient agar (Oxoid, UK) at 30 °C for 2 days. The DNA for a 16S rDNA sequencing analysis was prepared by the PrepMan method (Applied Biosystems, CA, USA), and the 16S rDNA sequence was determined with a MicroSeq 500 16S rDNA Kit (Applied Biosystems). The sequences of the other bacteria used for alignment and for calculating the homology levels were obtained from the MicroSeq Bacterial 500 Library Version 0023 (Applied Biosystems). MicroSeq Microbial Identification System Software Version 1.4.1 (Applied Biosystems) was used to align the sequences, and the phylogenetic distance was calculated by the neighbour-joining method. A morphological characterization of the isolated strain was performed with a nutrient agar medium (Oxoid, UK). The physiological characterization was performed according to the method of Barrow and Feltham [5]. Oxidase activity was assayed with the Cytochrome Oxidase Test Strip (Nissui Co., Tokyo, Japan).

2.4. Cultivation of strain Z-35-1

The strain was cultured in 5 ml of the N^{α}-Z-L-lysine medium, pH 7.0, at 30 °C for 2 days with shaking. The culture (1 ml) was then inoculated into the second medium (100/500 ml shaker flask), which was identical to the medium of the first culture. The second culture was incubated at 30 °C for 3 days with shaking (120 strokes/min). After cells were harvested from the second culture by centrifugation at 20,000 × *g* for 10 min, they were washed with 0.1 M potassium phosphate buffer, pH 7.0, and stored at −20 °C until use.

2.5. Standard reaction with cells

Twenty millimolars of N^{α}-Z-L-lysine or N^{α}-Z-D-lysine was incubated with cells from a 100 ml culture broth (approximately 35 mg in dry weight) at 30 °C for 4 days in 1.5 ml of 0.1 M potassium phosphate buffer, pH 7.0, with shaking (120 strokes/min). The reaction was terminated by separating the cells by centrifugation at 20,000 × *g* for 5 min, and the supernatant was used for assay of the reaction products.

2.6. Analysis of reaction products

The reaction products obtained by incubating N^{α}-Z-L-lysine or N^{α}-Z-D-lysine with cells were analyzed with TLC and HPLC under the same conditions as Isobe et al. [3]. The aldehyde group of reaction products was analyzed with 3-methyl-2-benzothiazolinone hydrazone (MBTH) according to the method described by Isobe et al. [3].

2.6.1. TLC method

TLC was carried out using Silica gel 60 (Merck, Darmstadt, Germany) and two solvents: solvent 1, ethanol:water (70:30), and solvent 2, phenol:water (75:25). After development of the samples, a 25% hydrogen bromide–acetic acid solution was sprayed on a TLC plate to detect an α-amino group. The products were detected with ninhydrin reagent.

2.6.2. HPLC method

2.6.2.1. TSK-Gel DEAE-5PW column. The reaction products were separated by a TSK-Gel DEAE-5PW column at a flow rate of 0.8 ml/min at 40 °C. The column was eluted with water for 5 min, followed by increasing the NaCl concentration to 0.3 M with a linear gradient for 10 min, and then by 0.3 M NaCl for 10 min. Detection was carried out at 210 nm.

2.6.2.2. Syn Pro Pep C_{18} column. The reaction products were separated by a Syn Pro Pep C_{18} column (Shimadzu, Kyoto, Japan) with an eluent of 0.1% trifluoroacetic acid for

5 min, followed by a linear gradient (0–45%) with 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile for 45 min, and then by 45% of 0.1% trifluoroacetic acid in acetonitrile for 5 min at a flow rate of 1.0 ml/min. Peaks were monitored at 210 nm.

2.6.3. Identification of aldehyde group

The eluate (50 μl) from HPLC was added to 0.2 M glycine-HCl buffer, pH 4.0 (0.75 ml), and mixed with 0.3 ml of 1.0% MBTH (derivative 1). Derivative 2 was prepared by adding 0.75 ml of 0.2% $FeCl_3$ solution to 0.2 ml of derivative 1.

3. Results

3.1. Isolation of microorganisms

In the first step, 200 bacterial strains were randomly isolated from 43 different soil samples after triple-enrichment culture with N^{α}-Z-L-lysine medium as described in Section 2. In the second step, 36 strains utilizing N^{α}-Z-L-lysine with high speed were selected by TLC analysis of the culture supernatant. The cells of the selected strains were then incubated with N^{α}-Z-L-lysine, and the reaction products were analyzed with TLC and HPLC. These analyses revealed that two strains formed N^{α}-Z-L-AAA, but the other 34 strains did not. In the two strains, strain Z-35-1 formed 0.61 mM N^{α}-Z-L-AAA by cells from 5 ml of culture broth, whereas the other strain formed 0.12 mM N^{α}-Z-L-AAA. Thus, the N^{α}-Z-L-AAA productivity of strain Z-35-1 was five times higher than that of the other strain. In addition, strain Z-35-1 also exhibited higher productivity of N^{α}-Z-D-AAA. Furthermore, this strain was easily cultivated in a liquid medium. We therefore selected strain Z-35-1 and used it in the following studies.

3.2. Identification of isolated strain

The isolated strain, Z-35-1, was identified by phylogenetic analysis and its biochemical characteristics. The 16S rDNA sequence of this strain showed a high similarity to a strain of *Rhodococcus erythropolis* (98.2%) and formed a sister group to that of *R. erythropolis* in the phylogenetic tree (data not shown). Thus, the isolated strain might belong to the genus *Rhodococcus* and probably belong to a group closely related to *R. erythropolis*, but may not be the same species as *R. erythropolis*. The biological traits of this strain were characterized as follows: The colony was circular, smooth and pale-yellow in color. The cell was rod-shaped (0.7–0.8 μm × 1.5–2.0 μm), nonmotile, Gram-positive and nonspore-forming. This strain was methophilic (grew at 37 °C, but not at 45 °C), catalase-positive, oxidase-negative, and nonfermentative. Since these biological traits also supported our assumption that the isolated strain belonged to the genus *Rhodococcus*, we named it *Rhodococcus* sp. AIU Z-35-1.

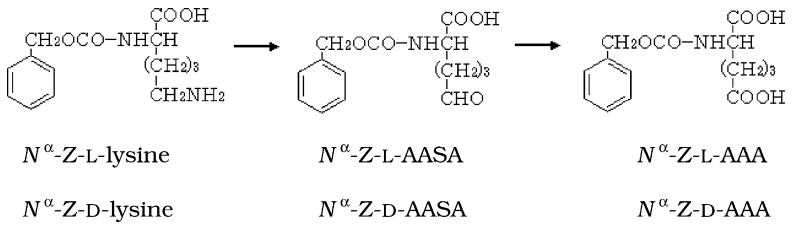
Table 1
Elution time and Rf values of reaction product and standard amino acids

Amino acid	Elution time (min)		Rf value	
	DEAE-5PW	C18	Solvent 1	Solvent 2
N^{α}-Z-L-lysine	3.4	27.2	0.77	0.50
N^{α}-Z-D-lysine	3.4	27.2	0.77	0.50
N^{α}-Z-L-AAA	21.4	32.8	0.92	0.35
N^{α}-Z-D-AAA	21.4	32.8	0.92	0.35
N^{α}-Z-L-AASA	24.1	–	–	–
N^{α}-Z-D-AASA	24.1	–	–	–
Reaction with N^{α}-Z-L-lysine				
Product-1	21.4	32.8	0.92	0.35
Product-2	24.1	–	–	–
Reaction with N^{α}-Z-D-lysine				
Product-1	21.4	32.8	0.92	0.35
Product-2	24.1	–	–	–

HPLC analyses were carried out under the conditions described in Section 2 using a TSK-Gel DEAE-5PW column and a Syn Pro Pep C_{18} column. TLC analyses were carried out using Silica gel 60. Solvent 1, ethanol:water (70:30). Solvent 2, phenol:water (75:25). The products were detected with ninhydrin reagent after 25% hydrogen bromide–acetic acid solution was sprayed on TLC plate and dried.

3.3. Reaction products from N^{α}-Z-L-lysine

Rhodococcus sp. AIU Z-35-1 was cultured in the N^{α}-Z-L-lysine medium at 30 °C for 3 days. Cells from the 100 ml culture broth were then incubated with 20 mM N^{α}-Z-L-lysine at 30 °C for 4 days under standard reaction conditions, and the reaction products were analyzed using the supernatant of the reaction mixture. HPLC analysis with a TSK-Gel DEAE-5PW column showed that the peak of N^{α}-Z-L-lysine (elution at 3.4 min) decreased, and two new peaks appeared at 21.4 and 24.1 min (Table 1). Since we had revealed that N^{α}-Z-L-AAA and N^{α}-Z-L-AASA were eluted at 21.4 and 24.1 min, respectively, by HPLC analysis with a TSK-Gel DEAE-5PW column [3,4], the solution eluted at 21.4 min was further analyzed by HPLC with a Syn Pro Pep C_{18} column. A single peak was detected at 32.8 min, which was also the same as the elution time of N^{α}-Z-L-AAA (Table 1). In TLC analysis of the solution eluted at 21.4 min, one spot of violet color was obtained by spraying a ninhydrin reagent after the TLC plate was treated with a hydrogen bromide–acetic acid solution, and its mobility was the same as that of N^{α}-Z-L-AAA in two solvent systems; the Rf values of 0.92 and 0.35 were obtained in solvents 1 and 2, respectively (Table 1). In addition, the solution eluted at 21.4 min did not exhibit a spectrum with an absorption maximum in the visible region by the reaction with MBTH and $FeCl_3$ (data not shown). Thus, one product from N^{α}-Z-L-lysine by the cell-reaction with *Rhodococcus* sp. AIU Z-35-1 was identified as N^{α}-Z-L-AAA. Since the elution time of another peak, 24.1 min, was the same as that of N^{α}-Z-L-AASA by HPLC analysis with a TSK-Gel DEAE-5PW column, the aldehyde group of this eluate was analyzed with MBTH. Derivative 2 with MBTH showed adsorption maxima at around 620 and 660 nm (adsorption spectra were the same as those of Ref. [3]), indicating that this product contained an aldehyde group. These results indicated that another



Scheme 1. Formation of N^{α}-Z-L-AAA and N^{α}-Z-D-AAA by the reaction with cells from *Rhodococcus* sp. AIU Z-35-1.

product from N^{α}-Z-L-lysine was N^{α}-Z-L-AASA. From these results, it was concluded that N^{α}-Z-L-lysine was converted into N^{α}-Z-L-AAA via N^{α}-Z-L-AASA by the reaction with cells from *Rhodococcus* sp. AIU Z-35-1 (Scheme 1).

3.4. Reaction products from N^{α}-Z-D-lysine

Fifty millimolars of N^{α}-Z-D-lysine was incubated with the cells from *Rhodococcus* sp. AIU Z-35-1 under the same conditions as those in the reaction with N^{α}-Z-L-lysine, and the reaction products were analyzed according to the above methods. The peak of N^{α}-Z-D-lysine eluting at 3.4 min decreased, and two new peaks appeared at 21.4 and 24.1 min by HPLC analysis with a TSK-Gel DEAE-5PW column (Table 1). Since each elution time of these new peaks was the same as that of N^{α}-Z-D-AAA and N^{α}-Z-D-AASA, respectively, the solutions of 21.4 and 24.1 min were further analyzed by the same methods used in the analyses of reaction products from N^{α}-Z-L-lysine. The solution eluted at 21.4 min from a TSK-Gel DEAE-5PW column was eluted at 32.8 min by HPLC with a Syn Pro Pep C_{18} column, and Rf values of 0.92 and 0.35 were obtained by TLC analysis (Table 1). The solution eluted at 24.1 min showed adsorption maxima at 620 and 660 nm in its derivative 2 with MBTH (data not shown). Thus, it was concluded that cells from *Rhodococcus* sp. AIU Z-35-1 also utilized N^{α}-Z-D-lysine, which was taken to be converted into N^{α}-Z-D-AAA via N^{α}-Z-D-AASA according to Scheme 1.

3.5. Effects of culturing time on N^{α}-Z-L-AAA formation

Rhodococcus sp. AIU Z-35-1 was cultured in the N^{α}-Z-L-lysine medium (100/500 ml shaker flask) at 30 °C for 6 days. The cells harvested from 100 ml culture broth at each day were incubated with 20 mM N^{α}-Z-L-lysine at 30 °C for 4 days, and the formation of N^{α}-Z-L-AAA was analyzed by HPLC with a TSK-Gel DEAE-5PW column. When cells cultured for one day were reacted with N^{α}-Z-L-lysine, the N^{α}-Z-L-AAA thus formed was less than 5% of the initial concentration of N^{α}-Z-L-lysine. However, cells cultured for 2 days completely utilized N^{α}-Z-L-lysine during a 4-day reaction, and approximately 65% of the initial concentration of N^{α}-Z-L-lysine was converted into N^{α}-Z-L-AAA. Furthermore, 74% of the initial concentration of N^{α}-Z-L-lysine was converted into N^{α}-Z-L-AAA by the reaction with cells cultured for 3 days. When cells cultured for more than 4 days were used, the utilization speed of N^{α}-Z-L-lysine became slower than that of the cells cultured for 3 days, and the conversion yield of N^{α}-Z-L-AAA also decreased (Table 2). Thus, cells cultured for 3 days proved to be the optimum for producing N^{α}-Z-L-AAA from N^{α}-Z-L-lysine.

Table 2
Effects of culturing time on N^{α}-Z-L-AAA production by reaction with cells from *Rhodococcus* sp. AIU Z-35-1

Culturing time (day)	Cell growth (OD660)	N^{α}-Z-L-lysine (mM)	N^{α}-Z-L-AAA (mM)	Conversion yield (%)
1	0.15	19.1	0.9	4.5
2	1.36	0	12.9	65
3	1.93	0	14.8	74
4	2.14	0	13.4	67
5	2.00	11.1	9.4	47
6	2.04	17.4	3.0	15

Cells from each day's 100 ml culture broth were incubated with 20 mM N^{α}-Z-L-lysine at 30 °C for 4 days under standard reaction conditions. Concentrations of N^{α}-Z-L-lysine and N^{α}-Z-L-AAA were obtained from peak area of HPLC analysis with a TSK-Gel DEAE-5PW column.

3.6. Production of N^{α}-Z-L-AAA

Using the cells cultured at 30 °C for 3 days, the optimum conditions for N^{α}-Z-L-AAA production were investigated. When pH of standard reaction conditions was varied between pH 5.5 and 8.5, the maximum production of N^{α}-Z-L-AAA was obtained at pH 7.0, and the product amount of N^{α}-Z-L-AAA decreased in the acidic and alkaline regions (Fig. 1). The optimum reaction temperature was investigated by incubating from 10 to 50 °C. The formation of N^{α}-Z-L-AAA increased until 30 °C, but decreased at higher temperatures (Fig. 2). The effect of N^{α}-Z-L-lysine concentration was investigated using 10–100 mM N^{α}-Z-L-lysine and cells of the 100 ml culture broth. The highest conversion yield of N^{α}-Z-L-AAA was obtained at 20 mM N^{α}-Z-L-lysine, while the product amounts of N^{α}-Z-L-AAA from 50 and 100 mM N^{α}-Z-L-lysine were higher than that from 20 mM N^{α}-Z-L-lysine (Table 3A). When the cells from 50 to 400 ml of culture broth were used, the product amounts of N^{α}-Z-L-AAA increased with increasing cell amounts, and reached a maximum with cells from a 200 ml culture broth (Fig. 3). On the basis of these results, 50 and 100 mM N^{α}-Z-L-lysines were incubated with cells of 200 ml culture broth in 0.1 M potassium phosphate buffer, pH 7.0, at 30 °C for 6 days. By incubation at 4 days, approximately 40 and 50 mM N^{α}-Z-L-AAA were pro-

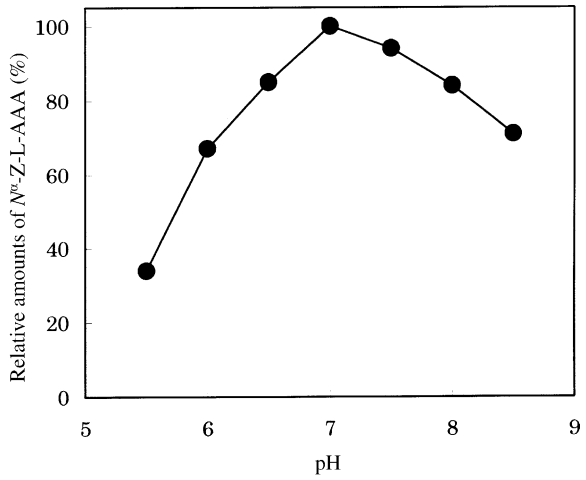


Fig. 1. Effects of pH on N^{α}-Z-L-AAA production by the reaction with cells from *Rhodococcus* sp. AIU Z-35-1. The reaction was carried out under standard reaction conditions, except that reaction pH varied between pH 5.5 and 8.5 by 0.1 M potassium phosphate buffer.

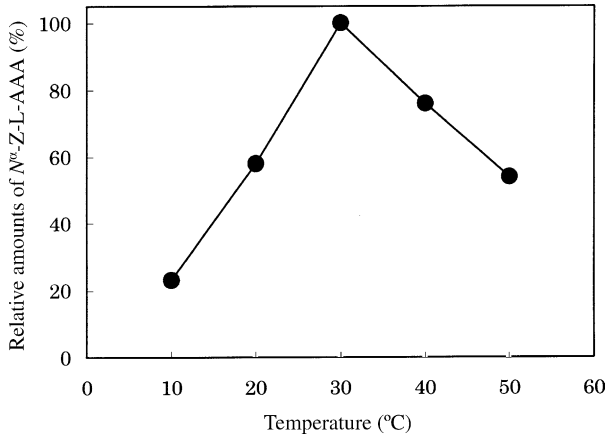


Fig. 2. Effects of temperature on N^{α}-Z-L-AAA production by the reaction with cells from *Rhodococcus* sp. AIU Z-35-1. The reaction was carried out under standard reaction conditions, except for the reaction temperature.

Table 3
Effects of substrate concentration on production of N^{α}-Z-L-AAA and N^{α}-Z-D-AAA by reaction with cells from *Rhodococcus* sp. AIU Z-35-1

N^{α}-Z-L-lysine (mM)	N^{α}-Z-L-AAA (mM)	Conversion yield (%)
(A)		
10	6.2	62
20	14.4	72
50	31.9	64
100	30.2	30
N^{α}-Z-D-lysine (mM)	N^{α}-Z-D-AAA (mM)	Conversion yield (%)
(B)		
20	14.6	73
50	38.1	76
100	44.9	45

(A) Cells of 100 ml culture broth were incubated with 10–100 mM N^{α}-Z-L-lysine at 30 °C for 4 days with shaking. (B) Cells of 200 ml culture broth were incubated with 20–100 mM N^{α}-Z-D-lysine at 30 °C for 4 days with shaking. Concentrations of N^{α}-Z-L-AAA and N^{α}-Z-D-AAA were calculated from peak area of HPLC analysis with a TSK-Gel DEAE-5PW column.

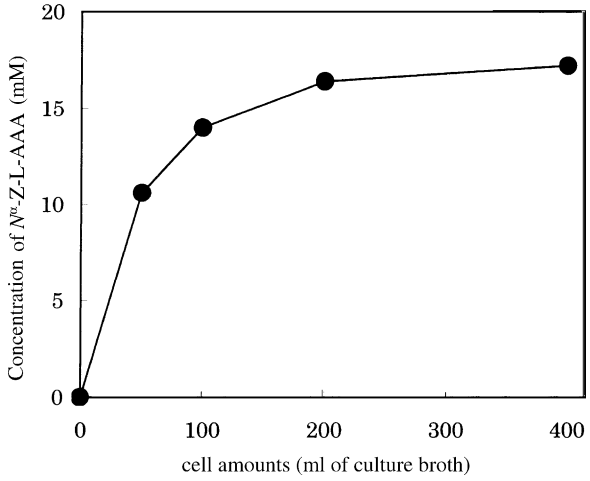


Fig. 3. Effects of cell concentration on N^{α}-Z-L-AAA production by the reaction with cells from *Rhodococcus* sp. AIU Z-35-1. The reaction was carried out under standard reaction conditions, except for the cell amounts.

duced from 50 and 100 mM N^{α}-Z-L-lysine, respectively, and their concentrations further increased to 42 and 56 mM, respectively, by prolonging the reaction time (Fig. 4A).

3.7. Production of N^{α}-Z-D-AAA

The effect of N^{α}-Z-D-lysine concentration on N^{α}-Z-D-AAA production was investigated by incubating 20–100 mM N^{α}-Z-D-lysine with cells from a 200 ml culture broth. The yield of N^{α}-Z-D-AAA from 20 and 50 mM N^{α}-Z-D-lysine was higher than that from 100 mM N^{α}-Z-D-lysine, while the product amount of N^{α}-Z-D-AAA from 100 mM N^{α}-Z-D-lysine was higher than those from 20 and 50 mM N^{α}-Z-D-lysine (Table 3B). Since these results were similar to those of N^{α}-Z-L-AAA production, 50 and 100 mM N^{α}-Z-D-lysines were incubated with cells from 200 ml of culture broth under the same conditions as those for N^{α}-Z-L-AAA production. The peak of N^{α}-Z-D-AAA eluted at 21.4 min increased with the reaction time, and more than 40 and 50 mM N^{α}-Z-D-AAA were produced from 50 and 100 mM N^{α}-Z-D-lysine, respectively, by incubation at 6 days (Fig. 4B). Since this strain converted N^{α}-Z-D-lysine into N^{α}-Z-D-AAA with a high conversion yield, it was also useful for the production of N^{α}-Z-D-AAA from N^{α}-Z-D-lysine.

4. Discussion

L-α-AAA is a precursor of β-lactam antibiotics, and the chemical methods for L-α-AAA and N^{α}-acetyl-L-AAA have been developed over a long time. However, they have remained very complicated and the recoveries have been low [6–8]. Since L-α-AAA and its related compounds have recently become increasingly important as raw materials for the chemical synthesis of new antibiotics or physiological peptides, biochemical methods for L-α-AAA production have

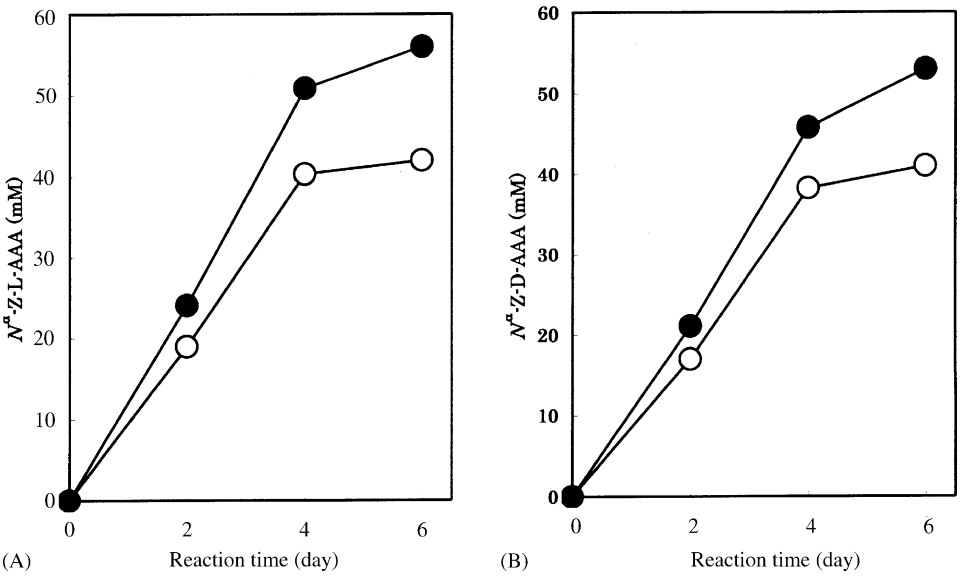


Fig. 4. Production of N^{α}-Z-L-AAA and N^{α}-Z-D-AAA by the reaction with cells from *Rhodococcus* sp. AIU Z-35-1 under optimum conditions, N^{α}-Z-L-Lysine and N^{α}-Z-D-lysine were incubated at pH 7.0 for 6 days at 30 °C with cells from 200 ml of culture broth. (A) N^{α}-Z-L-AAA production. (B) N^{α}-Z-D-AAA production. (◯) 50 mM of N^{α}-Z-L-lysine or N^{α}-Z-D-lysine was used as starting material; (●) 100 mM of N^{α}-Z-L-lysine or N^{α}-Z-D-lysine was used as starting material.

also been developed utilizing α-ketoadipate or L-lysine as the starting materials [1,2]. Fujii et al. obtained the genes of both L-lysine 6-aminotransferase and Δ-1-piperideine-6-carboxylate dehydrogenase from *Flavobacterium* [9,10], and constructed a recombinant strain for producing L-α-AAA from L-α-lysine [2]. This microbial method produced L-α-AAA during cultivation, but L-glutamic acid, $MgCl_2$ and $CaCl_2$ were added to increase the conversion yields. The method utilizing α-ketoadipate also used two substrates and two enzymes under the biphasic reaction, but it was not a simple method [1]. For the biochemical production of L-α-AAA derivatives, we have recently revealed that N^{α}-Z-L-AAA was formed from N^{α}-Z-L-lysine by the reaction with mycelia from *A. niger* AKU 3302, and we developed a new microbial method for N^{α}-Z-L-AAA production [3]. However, the formation speed and yield of N^{α}-Z-L-AAA by this method were not high, and little N^{α}-Z-D-AAA was formed. In addition, the cultivation of fungi was more difficult than that of bacteria in a liquid medium. Therefore, we have been screening new bacterial strains for producing N^{α}-Z-L-AAA and N^{α}-Z-D-AAA with a high yield. Here we report on a new strain belonging to the genus *Rhodococcus*, and on the development of a new microbial method for production of N^{α}-Z-L-AAA and N^{α}-Z-D-AAA. By this method, the formation speed and yield of N^{α}-Z-L-AAA have been improved. For example, 40 and 51 mM N^{α}-Z-L-AAA were produced from 50 and 100 mM N^{α}-Z-L-lysine, respectively, by incubating at 30 °C for 4 days. Thus, in the production of N^{α}-Z-L-AAA, the ability of a new isolated strain was more than 20 times higher than that of *A. niger* AKU 3302. In addition, the new isolated strain quickly utilized N^{α}-Z-D-lysine, and converted it into N^{α}-Z-D-AAA with a high yield, while little N^{α}-Z-D-AAA was produced by cell reaction with *A. niger* AKU 3302. Thus, a new isolated strain, *Rhodococcus* sp. AIU Z-35-1, was markedly superior to *A. niger* AKU 3302 for the production of N^{α}-Z-L-AAA and N^{α}-Z-D-AAA. In a future study, we intend to identify the enzymes catalyzing the conversion of N^{α}-Z-L-lysine and N^{α}-Z-D-lysine into N^{α}-Z-L-AAA and N^{α}-Z-D-AAA, respectively.

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