Journal of Molecular Catalysis B: Enzymatic 59 (2009) 47–51

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Microbial resolution of N^{ε}-acetyl-DL-lysine with *Rhodococcus* sp. AIU Z-35-1

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ARTICLE INFO

Article history:
Received 16 August 2008
Received in revised form 15 December 2008
Accepted 29 December 2008
Available online 8 January 2009

Keywords:
N^{α}-benzyloxycarbonyl-L-lysine
N^{α}-benzyloxycarbonyl-D-lysine
N^{ε}-acetyl-L-lysine
N^{ε}-acetyl-D-lysine
6-Acetylamino-2-oxohexanoic acid

ABSTRACT

An enantioselective resolution of N^{ε}-acetyl-DL-lysine was investigated using cells from *Rhodococcus* sp. AIU Z-35-1 cultivated with L-lysine medium. N^{ε}-Acetyl-L-lysine was deaminated by the resting cells, and 6-acetylamino-2-oxohexanoic acid was formed, but N^{ε}-acetyl-D-lysine was not. This reaction was optimum at pH 6.5 and 30 °C, and the highest reaction speed was obtained by cells harvested after 1 day of cultivation. When 150 mM N^{ε}-acetyl-DL-lysine was incubated at pH 6.5 and 30 °C for 5 days with cells harvested after 1 day of cultivation, more than 98% of the N^{ε}-acetyl-L-lysine was converted to 6-acetylamino-2-oxohexanoic acid, and N^{ε}-acetyl-D-lysine completely remained. Thus, N^{ε}-acetyl-D-lysine was enantioselectively produced from N^{ε}-acetyl-DL-lysine by the cell reaction with *Rhodococcus* sp. AIU Z-35-1. This cell reaction was also useful for the efficient production of 6-acetylamino-2-oxohexanoic acid from N^{ε}-acetyl-L-lysine.

1. Introduction

L-α-Aminoadipate δ-semialdehyde (L-α-AASA) and L-α-aminoadipic acid (L-α-AAA) are important precursors in biosynthesis of β-lactam antibiotics or L-lysine [1,2]. Since those precursors and their related compounds have become interesting raw materials for chemical synthesis of new antibiotics or physiologically active peptides, some chemical and biochemical methods for production of L-α-AASA, L-α-AAA or their derivatives have been developed [3–9]. However, those methods were complicated and their conversion yields were low. To overcome such drawbacks, we recently isolated a new bacterial strain, *Rhodococcus* sp. AIU Z-35-1, for production of N^{α}-benzyloxycarbonyl-L-amino adipic acid (N^{α}-Z-L-AAA) from N^{α}-benzyloxycarbonyl-L-lysine (N^{α}-Z-L-lysine). This strain converted N^{α}-Z-L-lysine to N^{α}-Z-L-AAA via N^{α}-benzyloxycarbonyl-L-aminoadipate-δ-semialdehyde (N^{α}-Z-L-AASA), and it was useful for selective production of N^{α}-Z-L-AASA and N^{α}-Z-L-AAA. Thus, N^{α}-Z-L-AASA and N^{α}-Z-L-AAA were efficiently produced by incubating at pH 5.0 with resting cells harvested after 1 day of cultivation, and by incubating at pH 7.0 with cells harvested after 3 days of cultivation, respectively [10,11]. When N^{α}-Z-D-lysine was used as substrate, N^{α}-Z-D-AASA and N^{α}-Z-D-AAA were also specifically produced under the same conditions as N^{α}-Z-L-AASA and N^{α}-Z-L-AAA, respectively [10,11]. These new microbial methods were markedly superior to chemical or other biochemical methods in both formation speed and yield of each reaction product. We further analyzed in detail the reactions of DL-lysine derivatives using resting cells from *Rhodococcus* sp. AIU Z-35-1, and obtained new findings that N^{ε}-acetyl-L-lysine is a good substrate of this strain, whereas N^{ε}-acetyl-D-lysine is not, although this strain utilized both N^{α}-Z-L-lysine and N^{α}-Z-D-lysine as a good substrate. These findings indicate that N^{ε}-acetyl-D-lysine is probably enantioselectively produced from N^{ε}-acetyl-DL-lysine by a cell reaction.

The present paper describes the optimum conditions for an efficient production of N^{ε}-acetyl-D-lysine from N^{ε}-acetyl-DL-lysine. The reaction product from N^{ε}-acetyl-L-lysine and the enzyme are also identified.

2. Materials and methods

2.1. Chemicals

N^{ε}-Acetyl-L-lysine and N^{ε}-acetyl-D-lysine were purchased from Watanabe Chemical Industries (Hiroshima, Japan). All other chemicals used were of analytical grade and were commercially purchased.

2.2. Cultivation of Rhodococcus sp. AIU Z-35-1

Rhodoccocus sp. AIU Z-35-1 was first cultivated in a test tube containing 5 ml of a L-lysine medium according to our previous report [12]. The culture broth (1.5 ml) was then inoculated into a 500 ml shaker flask containing 150 ml of the same medium and incubated at 30 °C for 1 day. Cells were harvested by centrifugation at 20,000 × *g* for 10 min, washed with 0.1 M potassium phosphate buffer, pH 7.0, and stored at −20 °C until use.

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doi:10.1016/j.molcatb.2008.12.019

2.3. Standard reaction with cells

Thirty micromoles of N^{ε}-acetyl-L-lysine or N^{ε}-acetyl-D-lysine was incubated with resting cells from 25 ml of culture broth (approximately 4.4 mg in dry weight) at 30 °C for 4 days in 1.5 ml of 0.3 M potassium phosphate buffer, pH 6.5, with shaking at 120 strokes per min. The reaction was terminated by separating the cells by centrifugation, and the supernatant was used for assay of reaction products.

2.4. HPLC analysis of reaction product

The reaction product was analyzed by HPLC with a TSK-Gel DEAE-5PW column (Tosoh, Tokyo, Japan). The elution was carried out at a flow rate of 0.8 ml per min at 40 °C with water for 5 min, followed by an increase of NaCl concentration to 0.3 M with a linear gradient for 10 min, and then by 0.3 M NaCl for 10 min. The amount of reaction product was calculated using the peak area of absorbance at 210 nm.

2.5. Identification of reaction product

The reaction product was separated by HPLC with a Syn Pro Pep RPC 18 column (Shimadzu, Kyoto, Japan) using an eluent of 0.1% trifluoroacetic acid (TFA) solution in water (A) and 0.1% TFA solution in acetonitrile (B). The HPLC was carried out with A for 5 min, followed by a linear gradient to 45% B at 45 min at 40 °C. The product was collected by monitoring absorbance at 210 nm, and the molecular mass was then analyzed by a HCT Ultra Mass Spectrometer (Bruker Daltonics GmbH, Bremen, Germany).

3. Results

3.1. Reaction of N^{ε}-acetyl-DL-lysine

The cells harvested after 1 day of cultivation (4.4 mg as a dried weight) were incubated with 30 mM N^{ε}-acetyl-L-lysine or 30 mM N^{ε}-acetyl-D-lysine under standard reaction conditions, and utilization of these compounds was analyzed by HPLC with a TSK-Gel DEAE-5PW column. The peak for N^{ε}-acetyl-L-lysine, which was eluted at 3.2 min, completely disappeared after 1 day of reaction, and one peak newly appeared at 15.6 min (data not shown). On the other hand, the peak for N^{ε}-acetyl-D-lysine did not decrease even after 2 days of reaction. These results indicate that this strain produced an enzyme utilizing N^{ε}-acetyl-L-lysine but not N^{ε}-acetyl-D-lysine. Thus, this strain has a possible application for biochemical resolution of N^{ε}-acetyl-DL-lysine.

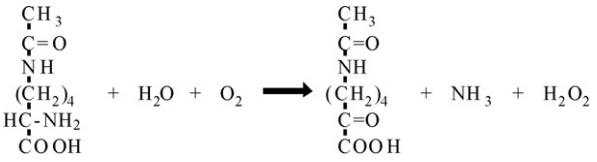
3.2. Identification of reaction product

The reaction product from N^{ε}-acetyl-L-lysine eluted at 15.6 min from a TSK-Gel DEAE-5PW column was then applied to a Syn Pro Pep RPC 18 column. The product was eluted at 4.3 min from the C_{18} column, and its molecular mass was estimated to be 187.08, indicating that N^{ε}-acetyl-L-lysine was converted to 6-acetylamino-2-oxohexanoic acid.

The formation of hydrogen peroxide was also confirmed by a color development method described in our previous report [12]. It was therefore concluded that N^{ε}-acetyl-L-lysine was converted to 6-acetylamino-2-oxohexanoic acid by an oxidative deamination reaction according to Scheme 1.

3.3. Identification of enzyme

To identify the enzyme catalyzing the conversion of N^{ε}-acetyl-L-lysine to 6-acetylamino-2-oxohexanoic acid by the cell reaction,



Scheme 1.

20 mM N^{ε}-acetyl-L-lysine or 20 mM N^{ε}-acetyl-D-lysine was incubated at 30 °C for 8 h with 0.2 unit of an L-amino acid oxidase purified from cells of *Rhodoccocus* sp. Z-35-1, since it has been revealed that the L-amino acid oxidase of this strain oxidized N^{ε}-acetyl-L-lysine, although the reaction product has not been identified [12]. The product of N^{ε}-acetyl-L-lysine by this reaction was eluted at 15.6 min by HPLC analysis with a TSK-Gel DEAE-5PW column (data not shown). These results indicate that the conversion of N^{ε}-acetyl-L-lysine to 6-acetylamino-2-oxohexanoic acid by the cell reaction was catalyzed by the L-amino acid oxidase produced in the cells.

3.4. Effects of reaction pH and temperature on deamination of N^{ε}-acetyl-L-lysine

Effects of pH on deamination of N^{ε}-acetyl-L-lysine by the cell reaction were analyzed under standard reaction conditions, except that the reaction pH was varied between pH 5.5 and 8.0. The reaction linearly proceeded for 12 h in these pH regions, and the reaction speed at pH 6.5 was faster than that at other pHs (Fig. 1).

Effects of reaction temperature on deamination of N^{ε}-acetyl-L-lysine were analyzed under standard reaction conditions, except that the reaction temperature was varied between 10 and 50 °C. The reaction linearly proceeded for 12 h below 40 °C, while the reaction at 50 °C stopped after 6 h of incubation. The product amount of 6-acetylamino-2-oxohexanoic acid at 30–40 °C was higher than that at the other temperatures after 24 h of incubation (Fig. 2).

From these results, it was concluded that the combination of pH 6.5 and 30 °C is optimum for conversion of N^{ε}-acetyl-L-lysine to 6-acetylamino-2-oxohexanoic acid.

3.5. Effect of cell age on deamination of N^{ε}-acetyl-L-lysine

Rhodococcus sp. AIU Z-35-1 was cultivated in a 500 ml shaker flask containing 100 ml of the L-lysine medium at 30 °C for 4 days.

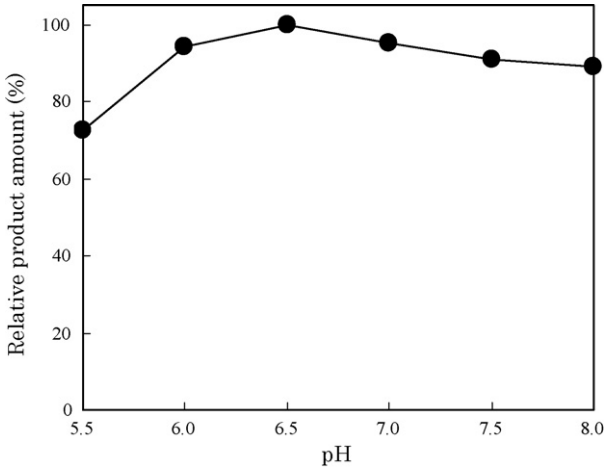


Fig. 1. Effect of reaction pH on deamination of N^{ε}-acetyl-L-lysine. Thirty micromoles of N^{ε}-acetyl-L-lysine was incubated with resting cells from 25 ml of culture broth (4.4 mg in dry weight) in 1.5 ml of reaction mixture. The reaction was carried out at 30 °C for 24 h at indicated pHs. The concentration of reaction product was analyzed by HPLC with a TSK-Gel DEAE-5PW column as described in Section 2. The relative amount of product (6-acetylamino-2-oxohexanoic acid) was calculated using conversion yields from N^{ε}-acetyl-L-lysine at each pH.

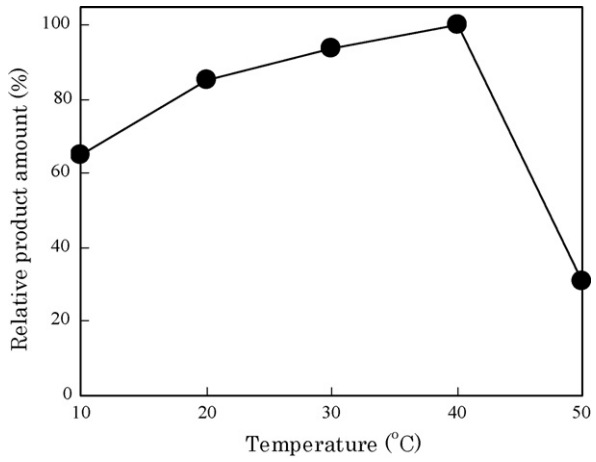


Fig. 2. Effect of reaction temperature on deamination of N^{ε}-acetyl-L-lysine. Thirty micromoles of N^{ε}-acetyl-L-lysine was incubated with resting cells from 25 ml of culture broth (4.4 mg in dry weight) in 1.5 ml of reaction mixture. The reaction was carried out at pH 6.5 for 24 h at indicated temperatures. The concentration of reaction product was analyzed by HPLC with a TSK-Gel DEAE-5PW column as described in Section 2. The relative amount of product (6-acetylamino-2-oxohexanoic acid) was calculated using conversion yields from N^{ε}-acetyl-L-lysine at each temperature.

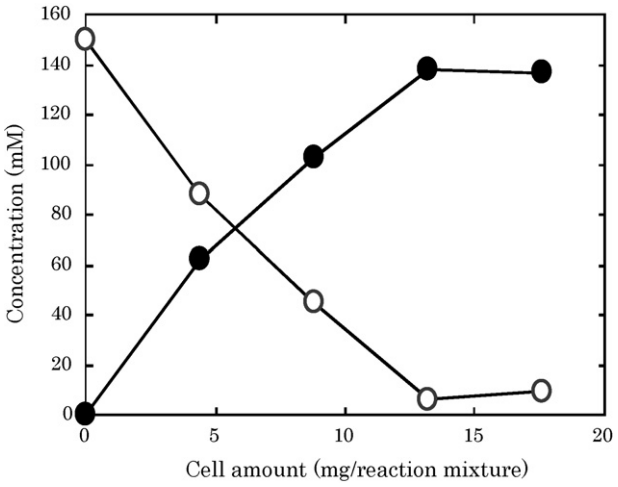


Fig. 3. Effect of cell amounts on deamination of N^{ε}-acetyl-L-lysine. Resting cells from 25 to 100 ml of culture broth (4.4–17.6 mg in dry weight) were incubated with 150 mM N^{ε}-acetyl-L-lysine at 30 °C and pH 6.5 for 4 days. The concentration of N^{ε}-acetyl-L-lysine (open circles) and 6-acetylamino-2-oxohexanoic acid (closed circles) was analyzed by HPLC with a TSK-Gel DEAE-5PW column as described in Section 2.

The cells from 25 ml of culture broth harvested each day were then incubated with 25 mM N^{ε}-acetyl-L-lysine at 30 °C and pH 6.5. When cells harvested after 1 day of cultivation were used, N^{ε}-acetyl-L-lysine was completely converted to 6-acetylamino-2-oxohexanoic acid during 24 h of reaction. When cells harvested after 2 days of cultivation were used, the conversion speed of N^{ε}-acetyl-L-lysine became slower than that of the cells harvested after 1 day of cultivation (Table 1). Thus, cells harvested after 1 day of cultivation proved to be optimal for conversion of N^{ε}-acetyl-L-lysine to 6-acetylamino-2-oxohexanoic acid.

3.6. Effects of substrate concentrations on deamination of N^{ε}-acetyl-L-lysine

When cells from 1 day of cultivation (4.4 mg as a dried weight) were incubated with 25–150 mM N^{ε}-acetyl-L-lysine at 30 °C and pH 6.5, 25 and 50 mM of N^{ε}-acetyl-L-lysine were completely converted to 6-acetylamino-2-oxohexanoic acid after 4 days of incubation, but 100 and 150 mM of N^{ε}-acetyl-L-lysine were not. In the case of 100 and 150 mM of N^{ε}-acetyl-L-lysine, approximately 70 mM of 6-acetylamino-2-oxohexanoic acid was produced after 4 days of incubation (Table 2). These results indicate that the above cell amounts or incubation time were not enough for complete deamination of 100 mM N^{ε}-acetyl-L-lysine within 4 days of reaction.

3.7. Effects of cell amounts on deamination of N^{ε}-acetyl-L-lysine

The cells from 25 to 100 ml of culture broth (4.4–17.6 mg as a dried weight) harvested after 1 day of cultivation were incubated with 150 mM N^{ε}-acetyl-L-lysine at 30 °C and pH 6.5. The N^{ε}-acetyl-L-lysine deamination became faster by increase of cell amounts up to 75 ml of culture broth (Fig. 3). When the reaction with cells from 50 ml of culture broth was prolonged until 6 days, most N^{ε}-acetyl-L-lysine was utilized, and the corresponding amount of 6-acetylamino-2-oxohexanoic acid was formed. These results indicate that the enzyme catalyzing this deamination reaction is stable, and a high concentration of N^{ε}-acetyl-L-lysine can be completely converted to 6-acetylamino-2-oxohexanoic acid by increasing the reaction time or cell amounts.

3.8. Resolution of N^{ε}-acetyl-DL-lysine

On the basis of the above results, 75 mM N^{ε}-acetyl-L-lysine, 75 mM N^{ε}-acetyl-D-lysine or 150 mM N^{ε}-acetyl-DL-lysine were incubated with cells from a 50 ml culture broth (8.8 mg as a dried weight) at pH 6.5 and 30 °C (Fig. 4). N^{ε}-Acetyl-L-lysine completely disappeared after 2 days of incubation, and a corresponding amount of 6-acetylamino-2-oxohexanoic acid was formed. On the other hand, utilization of N^{ε}-acetyl-D-lysine was not recognized even after 4 days of incubation. In the case of N^{ε}-acetyl-DL-lysine, the peak area of 3.2 min decreased to half of the original peak area, and the peak area appearing at 15.6 min was the same as that obtained from 75 mM N^{ε}-acetyl-L-lysine, although the reduction speed of a 3.2 min peak area and formation speed of a 15.6 min peak area were slower than those of 75 mM N^{ε}-acetyl-L-lysine. These results indicate that this strain is useful for enantioselective resolution of N^{ε}-acetyl-DL-lysine, although the deamination of N^{ε}-acetyl-L-lysine is slightly inhibited by N^{ε}-acetyl-D-lysine.

Table 1
Effect of cell age on utilization of N^{ε}-acetyl-L-lysine.

Cultivation time (h)	Growth (OD_{660})	Concentration after cell reaction	
		N^{ε}-Acetyl-L-lysine (mM)	6-Acetylamino-2-OHA (mM)
12	0.19	25.0	0
24	0.50	0.7	24.2
48	1.22	17.7	7.6
72	1.35	23.7	1.1

Rhodococcus sp. AIU Z-35-1 was cultivated in the L-lysine medium at 30 °C for indicated times with shaking (120 strokes/min). The resting cells from 25 ml of culture broth were incubated with 25 mM N^{ε}-acetyl-L-lysine at 30 °C for 24 h at pH 6.5 with shaking (120 strokes/min). The concentrations of N^{ε}-acetyl-L-lysine and 6-acetylamino-2-oxohexanoic acid (6-acetylamino-2-OHA) were analyzed by HPLC with a TSK-Gel DEAE-5PW column.

Table 2
Effect of substrate concentration on formation of 6-acetylamino-2-oxohexanoic acid.

N^{ε}-Acetyl-L-lysine (mM)	Concentration after cell reaction	
	N^{ε}-Acetyl-L-lysine (mM)	6-Acetylamino-2-OHA (mM)
25	0	25.0
50	0	50.0
100	32.0	68.7
150	80.6	70.0

The cells from 25 ml of culture broth harvested after 1 day of cultivation were incubated with 25–150 mM N^{ε}-acetyl-L-lysine at 30 °C for 4 days at pH 6.5 with shaking (120 strokes/min). The concentrations of N^{ε}-acetyl-L-lysine and 6-acetylamino-2-oxohexanoic acid (6-acetylamino-2-OHA) were analyzed by HPLC with a TSK-Gel DEAE-5PW column.

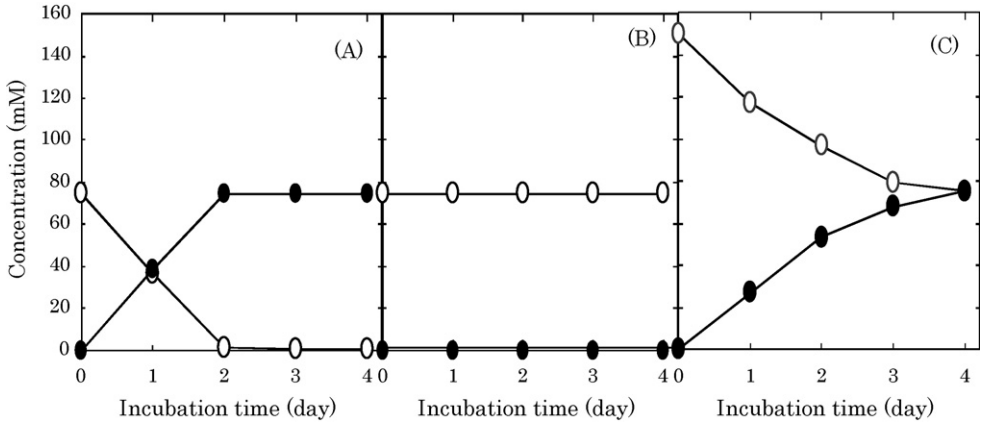


Fig. 4. Reaction of N^{ε}-acetyl-L-lysine, N^{ε}-acetyl-D-lysine and N^{ε}-acetyl-DL-lysine with resting cells from *Rhodoccocus* sp. AIU Z-35-1. Resting cells from 50 ml of culture broth (8.8 mg in dry weight) were incubated with 75 mM N^{ε}-acetyl-L-lysine (A), 75 mM N^{ε}-acetyl-D-lysine (B) and 150 mM N^{ε}-acetyl-DL-lysine (C) at 30 °C and pH 6.5 for 4 days. The concentration of N^{ε}-acetyl-L-lysine, N^{ε}-acetyl-D-lysine and reaction product was analyzed by HPLC with a TSK-Gel DEAE-5PW column as described in Section 2. Open circles, substrate; closed circles, 6-acetylamino-2-oxohexanoic acid (reaction product).

4. Discussion

Although we have already reported that *Rhodoccocus* sp. AIU Z-35-1 isolated in our laboratory could convert N^{α}-Z-L-lysine to N^{α}-Z-L-AASA or N^{α}-Z-L-AAA, and N^{α}-Z-D-lysine to N^{α}-Z-D-AASA or N^{α}-Z-D-AAA [10,11], we recently obtained new findings that this strain can utilize N^{ε}-acetyl-L-lysine but not N^{ε}-acetyl-D-lysine. We therefore identified products and enzyme in the cell reaction with N^{ε}-acetyl-L-lysine. In this cell reaction, N^{ε}-acetyl-L-lysine was converted to 6-acetylamino-2-oxohexanoic acid. When the L-amino acid oxidase purified from *Rhodoccocus* sp. AIU Z-35-1 [12] was incubated with N^{ε}-acetyl-L-lysine, 6-acetylamino-2-oxohexanoic acid was also identified as the reaction product. It was therefore concluded that the conversion of N^{ε}-acetyl-L-lysine to 6-acetylamino-2-oxohexanoic acid by the cell reaction was catalyzed by the L-amino acid oxidase produced by the strain, and the L-amino acid oxidase has a possibility of 6-acetylamino-2-oxohexanoic acid production from N^{ε}-acetyl-L-lysine.

In general, production by cell reaction is better than enzymatic methods, since the latter methods often require purified enzymes and catalase for efficient conversion of a high concentration of substrate. We therefore revealed optimum conditions for production of 6-acetylamino-2-oxohexanoic acid by the cell reaction, and resolution of N^{ε}-acetyl-DL-lysine was carried out under the optimum conditions. A high conversion yield of 6-acetylamino-2-oxohexanoic acid was obtained by the combination of pH 6.5 and 30 °C. This reaction efficiently proceeded using the cells harvested after 1 day of cultivation in a L-lysine medium. On the basis of these results, 150 mM N^{ε}-acetyl-DL-lysine was incubated with resting cells under the optimal conditions. N^{ε}-Acetyl-L-lysine was smoothly converted to 6-acetylamino-2-oxohexanoic acid, and $N\varepsilon$-acetyl-D-lysine remained without deaminating. Thus, the cells of *Rhodoccocus* sp. AIU Z-35-1 cultivated in the L-lysine medium were useful for the efficient production of N^{ε}-acetyl-D-lysine and 6-acetylamino-2-oxohexanoic acid from N^{ε}-acetyl-DL-lysine, whereas this strain could not be applied to an enantioselective resolution of N^{α}-acyl-DL-lysine [10,11]. These results indicate that *Rhodoccocus* sp. AIU Z-35-1 can produce an enzyme catalyzing deamination of the α-amino group of N^{ε}-acyl-L-lysine, but not an enzyme catalyzing deamination of the α-amino group of N^{ε}-acyl-D-lysine.

At present, some D-amino acids and their derivatives are produced by the enzymatic method using microbial D-aminoacylases [13,14]. However, the substrate specificity of those enzymes is limited. For production of a wide variety of D-amino acids, an enzyme with broad substrate specificity is preferable. Strains from *Rhodoccocus* sp. AIU Z-35-1 [12] or *R. opacus* DSM 43250 [15] reportedly produced an L-amino acid oxidase with broad substrate specificity. The present studies further indicate that cells or L-amino acid oxidase from *Rhodoccocus* sp. Z-35-1 are applicable to the selective production of N^{ε}-acyl-D-lysine. In a forthcoming study, we intend to investigate the application of *Rhodoccocus* sp. Z-35-1 cells or L-amino acid oxidase to the resolution of other DL-amino acids.

Acknowledgments

The authors wish to thank Prof. Yasuhisa Asano and Dr. Ken-ichi Fuhshuku, Toyama Prefectural University, for their analysis of the molecular mass of the reaction product.

References

[1] J.F. Martin, Appl. Microbiol. Biotechnol. 50 (1998) 1–15.
[2] D.E. Ehmann, A.M. Gehring, C.T. Walsh, Biochemistry 38 (1999) 6171–6177.
[3] J.R. Matos, C.-H. Wong, J. Org. Chem. 51 (1986) 2388–2389.
[4] D.H.R. Barton, Y. Herve, P. Potier, J. Thierry, Tetrahedron 43 (1987) 4297–4308.
[5] T. Hammer, R. Bode, Zentralbl. Microbiol. 147 (1992) 65–70.

[6] A. Rumbero, J.F. Martin, M.A. Lumbreras, P. Liras, C. Esmahan, Bioorg. Med. Chem. 3 (1995) 1237–1240.
[7] H. Agematsu, T. Fujii, Bio Industry 19 (2002) 40–47.
[8] K. Isobe, K. Tokuta, Y. Narita, A. Matsuura, T. Sakaguchi, N. Wakao, J. Mol. Catal. B, Enzyme 30 (2004) 119–123.
[9] K. Isobe, K. Tokuta, Y. Narita, A. Matsuura, T. Sakaguchi, N. Wakao, J. Mol. Catal. B, Enzyme 30 (2004) 13–18.
[10] K. Isobe, K. Tokuta, Y. Narita, A. Matsuura, T. Sakaguchi, N. Wakao, J. Mol. Catal. B, Enzyme 32 (2004) 27–32.
[11] K. Isobe, S. Nagasawa, K. Tokuta, A. Matsuura, T. Sakaguchi, N. Wakao, J. Biosci. Bioeng. 100 (2005) 288–291.
[12] K. Isobe, S. Nagasawa, J. Biosci. Bioeng. 104 (2007) 218–223.
[13] M. Moriguchi, K. Sakai, Y. Miyamoto, M. Wakayama, Biosci. Biotech. Biochem. 57 (1993) 1149–1152.
[14] S. Kumagai, M. Kobayashi, S. Yamaguchi, T. Kanaya, R. Motohashi, K. Isobe, J. Mol. Catal. B, Enzyme 30 (2004) 159–165.
[15] B. Geueke, W. Hunmmel, Enzyme Microb. Technol. 31 (2002) 77–87.