APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1988, p. 984–989
0099-2240/88/040984-06$02.00/0

Vol. 54, No. 4

Production and Purification of D-Aminoacylase from *Alcaligenes denitrificans* and Taxonomic Study of the Strain

YING-CHIEH TSAI,* CHING-PING TSENG, KUANG-MING HSIAO, AND LING-YUN CHEN

Institute of Biochemistry, National Yang-Ming Medical College, Taipei, Taiwan 11221, Republic of China

Received 15 July 1987/Accepted 11 January 1988

A D-aminoacylase-producing microorganism, strain DA181, isolated from soil was identified as *Alcaligenes denitrificans* subsp. *denitrificans*. This strain produced about 29,300 units (micromoles of product formed per hour) of D-aminoacylase and 2,300 units of L-aminoacylase per gram of cells (wet weight) when cultivated in a medium containing 1% *N*-acetyl-DL-leucine as the carbon source. The D-aminoacylase was purified 345-fold. The specific activity of the purified enzyme was 108,600 units per mg of protein when *N*-acetyl-D-methionine was used as a substrate. The apparent molecular weight was 58,000, as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *N*-Acetyl-D-methionine was the favored substrate, followed by *N*-acetyl-D-phenylalanine. This enzyme had a high stereospecificity, and its hydrolysis of *N*-acetyl-L-amino acids was almost negligible.

D-Amino acids such as D-valine and D-phenylglycine are intermediates for the preparation of various pesticides, bioactive peptides, and antibiotics (1, 13, 14). Several methods have been reported for the optical resolution of DL-amino acids into D-amino acids. Chemically synthesized *N*-acyl-DL-amino acids were optically resolved with L-aminoacylase to generate L-amino acids; the nonhydrolyzed *N*-acyl-D-amino acids were then chemically hydrolyzed to obtain D-amino acids. This method was developed mainly for the purpose of producing L-amino acids; the optical purity and recovery of the D-amino acid by-products were therefore not satisfactory. Takahashi et al. (17) developed another method for producing D-amino acids from position 5-substituted hydantoins, the intermediary products of a conventional process for amino acid synthesis. Hydantoins were asymmetrically hydrolyzed by a hydantoinase of *Pseudomonas striata* to give *N*-carbamyl-D-amino acids, which were then hydrolyzed by acid to produce D-amino acids.

The approach of using L-aminoacylase for the production of L-amino acids has been one of the most successful industrial applications of immobilized enzyme technology (2, 19–21). According to the same principle, a D-aminoacylase with strict stereospecificity should be a promising tool for the manufacture of D-amino acids. Microbial D-aminoacylases have been reported by Kameda et al. (6, 7) and Fukagawa and colleagues (5, 9, 10) in *Pseudomonas* species and by Sugie and Suzuki (15, 16) in *Streptomyces olivaceus*. The disadvantage of these D-aminoacylases was that their stereospecificities were not strict enough for some amino acids. This drawback restricted the use of these enzymes for the production of D-amino acids. We conducted a screening program for D-aminoacylase-producing microorganisms from soil. Strain DA181, identified as *Alcaligenes denitrificans* subsp. *denitrificans*, was isolated. The D-aminoacylase produced by this strain was shown to possess a high stereospecificity. Here we report the production, purification, and some properties of this D-aminoacylase and results of taxonomic studies of strain DA181.

* Corresponding author.

MATERIALS AND METHODS

Chemicals. *N*-Acetyl-D-methionine and *N*-acetyl-DL-methionine, -tryptophan, -valine, and -phenylalanine were kindly provided by Amano Pharmaceutical Co. Ltd. (Nagoya, Japan). D-, L-Amino acids, other *N*-acetyl-D-, L- and DL-amino acids, *N*-chloroacetyl-D-, L-valine, and 2,4,6-trinitrobenzenesulfonic acid (TNBS) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Molecular weight standards were from Bio-Rad Laboratories (Richmond, Calif.). All other chemicals were of analytic reagent grade.

Microorganisms and culture conditions. D-Aminoacylase-producing microorganisms were isolated by the following procedure. Soil extract was spread onto agar plates containing isolation medium, which was composed of 1% *N*-acetyl-D-methionine (the sole carbon and nitrogen source), 0.1% K_2HPO_4, and 0.05% $MgSO_4 \cdot 7H_2O$ (pH 7.0), and was solidified with 1.5% agar. Strains able to grow on this medium were selected and cultivated at 30°C in 20 ml of liquid isolation medium supplemented with 0.5% yeast extract in a 200-ml flask with constant shaking. The cells were collected by centrifugation, and the intracellular D-aminoacylase activity was measured after the cells were treated with toluene. A bacterial strain, named DA181, was selected.

For enzyme production, strain DA181 was cultivated in 2.5 liters of LYP medium (1% *N*-acetyl-DL-leucine, 0.5% yeast extract, 0.5% peptone, 0.1% K_2HPO_4, and 0.05% $MgSO_4 \cdot 7H_2O$ [pH 8.0]) at 30°C in a 5-liter mini jar fermentor (Mizuwa Rikagaku Kogyo Co., Osaka, Japan). The D-aminoacylase activity was analyzed after the cells were treated with toluene. LYP medium was also used for the stock slant culture of strain DA181.

Characterization and identification of strain DA181. Taxonomic characteristics of strain DA181 were investigated by the procedures described in *Manual of the Identification of Medical Bacteria*, 2nd ed. (3); *The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria* (18); and *Bergey's Manual of Systematic Bacteriology*, vol. 1 (8).

Toluene treatment of cells. Cells were suspended in 0.5 ml of ice-cold 50 mM Tris hydrochloride buffer (buffer A; pH 7.8), and then 0.2 ml of ice-cold toluene was added and

mixed thoroughly. After 20 min on ice, the cells were collected by centrifugation and suspended in 0.5 ml of buffer A, and the intracellular D-aminoacylase activity was determined.

Enzyme assay. The D-aminoacylase activity was measured by reacting TNBS with the D-methionine released from *N*-acetyl-D-methionine, which was shown to be the most preferable substrate for this enzyme (4). An appropriate amount of enzyme was incubated in 0.5 ml of buffer A containing 20 mM *N*-acetyl-D-methionine at 37°C for 20 min. The reaction was stopped by heating the mixture in boiling water for 5 min; 0.5 ml of 0.1 M $Na_2B_4O_7$ in 0.1 M NaOH and 20 μl of 1.1 M TNBS were then added, and the reaction mixture was incubated at 37°C for 5 min. A 2-ml portion of the Na_2SO_3 solution which was freshly prepared by mixing 1.5 ml of 0.1 M Na_2SO_3 with 98.5 ml of 0.1 M NaH_2PO_4, was then added. The A_{420} was determined. L-Aminoacylase activity was estimated by the same method, except that *N*-acetyl-L-methionine was used as the substrate.

One unit of enzyme activity was defined as the amount of enzyme which catalyzed the formation of 1 μmol of D- or L-methionine per h at 37°C.

Protein determination. Protein was determined by the method of Lowry et al. (12) by using bovine serum albumin as a standard.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out in a 5 to 20% linear gradient gel as described by Laemmli (11). Protein was detected by staining the gel with 0.004% Coomassie brilliant blue in 5% isopropanol–10% acetic acid.

Purification of the enzyme. A typical purification scheme of the D-aminoacylase from the cell extract of *A. denitrificans* subsp. *denitrificans* DA181 is described below. All operations were carried out at 4°C, and the columns used were preequilibrated with buffer A.

(i) Preparation of cell extract. Cells (75 g) collected from 9 liters of culture broth were suspended in 600 ml of buffer A containing 2 mM β-mercaptoethanol, the cells were sonicated, and the cell debris was removed by centrifugation.

(ii) Ammonium sulfate fractionation. Solid ammonium sulfate was added to the clarified cell extract with stirring to give 30% saturation. After the solution was allowed to stand for 1 h, the supernatant was collected by centrifugation and was brought to 70% ammonium sulfate saturation in the same manner. After the solution stood for 2 h, the precipitate was collected by centrifugation. It was dissolved in a minimal volume of buffer A and dialyzed against the same buffer.

(iii) DEAE-Sephadex A-50 column chromatography. The 30 to 70% ammonium sulfate fraction was applied to a DEAE-Sephadex A-50 column (9.0 by 35 cm). After the solution was washed with buffer A, the enzyme was then eluted with the same buffer containing 0.3 M NaCl at a flow rate of 120 ml/h. The active fractions were pooled and concentrated by lyophilization.

(iv) Sephadex G-100 column chromatography. The active enzyme fraction from DEAE-Sephadex A-50 column was applied to a Sephadex G-100 column (6.0 by 130 cm). The column was eluted with buffer A at a flow rate of 60 ml/h.

(v) Fractogel TSK DEAE-650 column chromatography. The pooled enzyme fraction from the Sephadex G-100 column was applied to a Fractogel TSK DEAE-650 column (2.5 by 75 cm). After the solution was washed with buffer A, it was washed with a 1.2-liter linear gradient of 0 to 0.2 M NaCl in buffer A. The flow rate was 40 ml/h. The active fractions were pooled and concentrated by lyophilization.

(vi) Second Sephadex G-100 column. The concentrated enzyme fraction was applied to a Sephadex G-100 column (2.5 by 105 cm) and eluted with buffer A at a flow rate of 13.6 ml/h. The active fractions were pooled and concentrated by lyophilization.

(vii) Fractogel TSK HW-50 column. The concentrated enzyme fraction was further purified by passing it through a gel filtration column of Fractogel TSKHW-50 (1.0 by 90 cm). The column was eluted with buffer A at a flow rate of 3.6 ml/h. The active fractions were pooled and concentrated by lyophilization.

(viii) SynChropak AX300 high-performance liquid chromatography. The concentrated enzyme fraction was dialyzed against 20 mM Tris hydrochloride buffer (pH 7.8). A 100-μl portion of the desalted enzyme solution containing of 1.2 mg of protein was injected to a weak anion-exchanger, high-performance liquid chromatographic column (SynChropak AX300, 250 by 4.6 mm; (SynChrom. Inc., Lafayette, Ind.) that was preequilibrated with 20 mM Tris acetate buffer (pH 7.8). The enzyme was eluted with a linear gradient of 0.2 to 0.4 M sodium acetate in 20 mM Tris acetate buffer (pH 7.8) for 40 min at a flow rate of 1.5 ml/min. The average operating pressure was 800 lb/in^2.

RESULTS

Characterization of the D-aminoacylase-producing bacterium strain DA181. Strain DA181 was isolated from the soil as described above. This strain was a gram-negative, rod-shaped (0.5 to 1.0 by 0.6 to 2.0 μm), obligately aerobic, motile bacterium with peritrichous flagella. This strain reacted positively to the following tests: nitrate reduction to nitrite, nitrate reduction to gas, oxidase, catalase, and accumulation of poly-β-hydroxybutyrate. It reacted negatively to following tests: urease; lecithinase; ornithine decarboxylase; lysine decarboxylase; tryptophan deaminase; phenylalanine deaminase; Voges-Proskauer; oxidation-fermentation; indol formation; H_2S formation; mucoid formation; dehydroacetone formation; hydrolysis of casein, chitin, cellulose, agar, gelatin, starch, Tween 80, and DNA; and growth in medium containing 7% NaCl. This strain was able to grow with acetate and D- or L-leucine as the sole carbon source, but not with glucose, fructose, xylose, arabinose, maltose, lactose, sucrose, trehalose, melobiose, cellobiose, sorbitol, mannitol, salicine, gluconic acid, malonic acid, D- or L-valine, methionine, or phenylalanine. The temperature range for growth was 9 to 40°C, and the pH range was 4.8 to 9.6. All properties tested were in good agreement with those for *A. denitrificans* subsp. *denitrificans* (3, 8, 18). A transmission electron micrograph of this strain is shown in Fig. 1.

Effect of medium pH on the production of D-aminoacylase. The effect of the initial pH of the medium on the production of D-aminoacylase was examined by using a medium containing 1% *N*-acetyl-D-methionine, 0.5% yeast extract, 0.5% peptone, 0.1% K_2HPO_4, and 0.05% $MgSO_4 \cdot 7H_2O$. The highest enzyme production was obtained with a starting pH of 8.0 (Fig. 2). When the initial pHs were between 5.0 and 10.0, the final pHs of the medium after 24 h of cultivation were from 8.5 to 9.0.

Effect of *N*-acetyl-amino acids on the production of D-aminoacylase. The *N*-acetyl-amino acids were used as nutrients for growth and as inductive reagents for enzyme production. The effects of various *N*-acetyl-amino acids at a concentration of 1% was investigated (Table 1). The addition of various *N*-acetyl-amino acids was shown to stimulate cell growth in all cases; and with the exception of *N*-acetyl-D- or

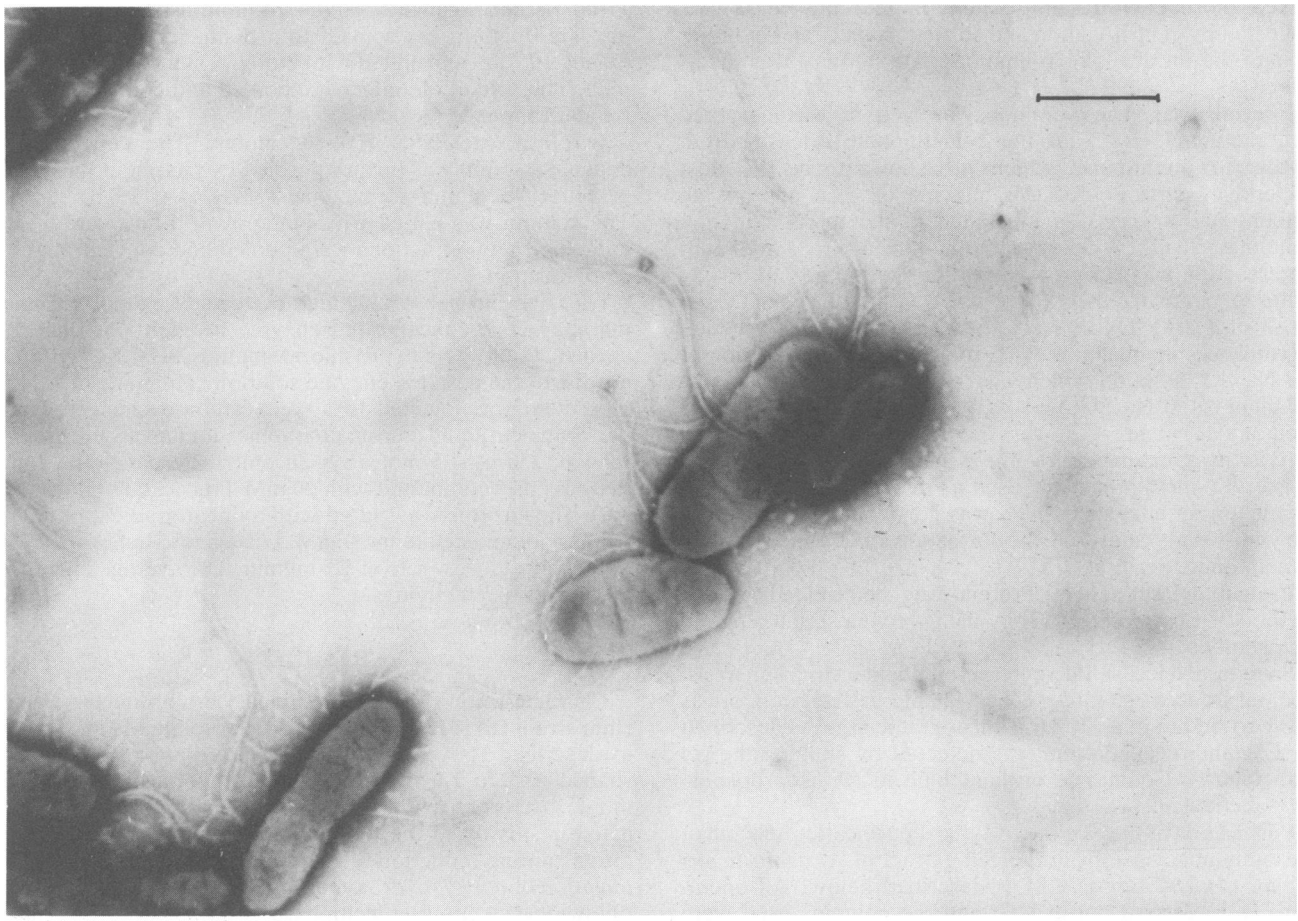

FIG. 1. Transmission electron micrograph of *A. denitrificans* DA181. Bar, 1.0 μm.

DL-tryptophan, these *N*-acetyl-amino acids greatly stimulated enzyme production. *N*-Acetyl-D-methionine and *N*-acetyl-D-phenylalanine worked better than *N*-acetyl-DL-methionine and *N*-acetyl-DL-phynylalanine, respectively, for enzyme induction. Among the tested compounds, *N*-acetyl-DL-leucine was the best for either enzyme production or cell growth and was selected for the following studies. The addition of 1% *N*-acetyl-DL-leucine increased the enzyme production five fold and stimulated cell growth two fold. The noticeable stimulatory effect of *N*-acetyl-DL-leucine on cell growth could be explained since this strain was able to utilize leucine, as well as acetate, as the sole carbon source; but it was not able to utilize the other amino acids tested. The optimum concentration of *N*-acetyl-DL-leucine for both enzyme production and cell growth was 2% (Fig. 3). A concentration of 1% was chosen for the rest of the study to reduce costs.

Time course of D-aminoacylase production. A typical time course of the cultivation of strain DA181 in a 5-liter bench-top jar fermentor is shown in Fig. 4. D-Aminoacylase activity reached 250 units per ml (equivalent to 29,300 units per g of cells) after 18 h of cultivation. Under these fermentation conditions, it produced about 20 units per ml, or 2,300 units of L-aminoacylase activity per g of cells. At the end of the fermentation, the pH of the broth increased to about 9.0 from a starting pH of 8.0.

Purification of D-aminoacylase. The purification of D-aminoacylase is summarized in Table 2. A 345-fold purification

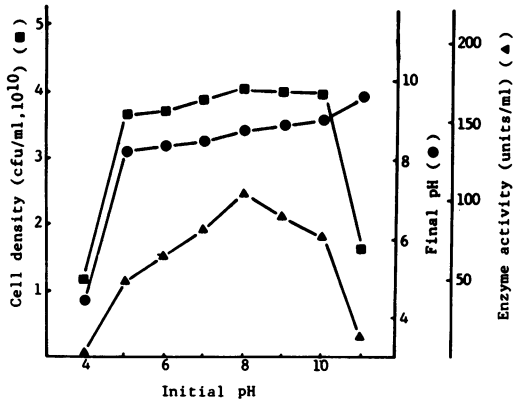

FIG. 2. Effect of medium pH on the production of D-aminoacylase. Strain DA181 was grown in a medium containing 1% *N*-acetyl-D-methionine, 0.5% yeast extract, 0.5% peptone, 0.1% K_2HPO_4, and 0.05% $MgSO_4 \cdot 7H_2O$ (pH 7.0) for 24 h at 30°C. The culture was then inoculated 2% into 500-ml shaking flasks, each of which contained 100 ml of the medium described above adjusted to the designated pH. The initial cell density was about 7×10^8 CFU/ml. After 24 h of culture, the pH of the culture broth (●), cell density (■), and D-aminoacylase activity in the toluene-treated cells (▲) were determined. The cell density was determined by determining the A_{550}, which was then calibrated from the CFU plate count versus absorbance curve.

TABLE 1. Effect of *N*-acetyl-amino acids on the production of D-aminoacylase[a]

N-Acetyl-amino acid	Relative activity (%)[b]	Cell growth (A_{550})
None	100	2.74
N-Acetyl-DL-leucine	498	4.92
N-Acetyl-D-valine	518	3.29
N-Acetyl-DL-valine	311	3.17
N-Acetyl-D-phenylalanine	418	3.01
N-Acetyl-DL-phenylalanine	286	3.11
N-Acetyl-D-methionine	242	3.37
N-Acetyl-DL-methionine	143	3.21
N-Acetyl-D-tryptophan	114	3.63
N-Acetyl-DL-tryptophan	102	3.47
N-Acetyl-D-phenylglycine	387	3.21

[a] Strain DA181 was grown in 100 ml of medium containing 1% *N*-acetyl-amino acids, 0.5% yeast extract, 0.5% peptone, 0.1% K_2HPO_4, and 0.05% $MgSO_4 \cdot 7H_2O$ (pH 8.0) in 500-ml shaking flasks at 30°C. The initial cell density was about 7×10^8 CFU/ml. After 24 h of cultivation, the cell growth and the intracellular D-aminoacylase activity were determined.

[b] The activity obtained in the medium without *N*-acetyl-amino acids was about 30 units per ml, which was assigned a value of 100.

was obtained, with a recovery of 11%. The specific activity of the purified enzyme was 108,600 units per mg of protein. The L-aminoacylase present in the crude extract was eliminated by the first DEAE-Sephadex column chromatography (Fig. 5). The enzyme activity eluted from the Fractogel HW-50 column was a symmetrical peak that coincided with a major protein peak (data not shown). When this enzyme preparation was chromatographed by high-performance liquid chromatography, several minor contaminating proteins could be further separated from the enzyme peak (Fig. 6). The specific activity of the enzyme increased significantly after this step.

Purity and molecular weight of the enzyme. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the enzyme preparation purified by high-performance liquid chromatography showed the presence of trace amounts of a contaminant protein with a faster mobility, which could be removed by rechromatography on a high-performance liquid chromatograph (Fig. 7). By using molecular weight standards, the molecular weight of the enzyme was estimated to be 58,000.

Substrate specificity. The relative activity of the purified D-aminoacylase toward various *N*-acetyl-D- or L-amino acids is summarized in Table 3. *N*-Acetyl-D-methionine was the favored substrate; the next most favored substrate was *N*-acetyl-D-phenylalanine, followed by *N*-chloroacetyl-D-valine. *N*-Acetyl-D-valine, in contrast, was a poor substrate. Of all the *N*-acetyl-L-amino acids tested, the relative activities were all less than 1%.

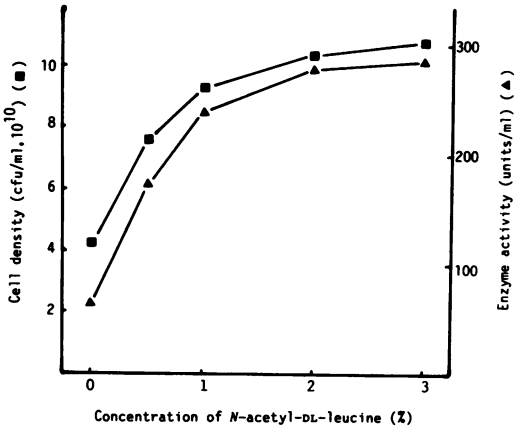

FIG. 3. Effect of *N*-acetyl-DL-leucine concentration on the production of D-aminoacylase. Strain DA181 was grown in LYP medium for 24 h at 30°C. The culture was then inoculated 2% into 500-ml flasks, each of which contained 100 ml of LYP medium with various concentrations of *N*-acetyl-DL-leucine. The initial cell density was about 1.2×10^9 CFU/ml. After 24 h of culture, the cell density (■) and the D-aminoacylase activity in the toluene-treated cells (▲) were determined. The cell density was determined by determining the A_{550}, which was then calibrated from the CFU plate count versus absorbance curve.

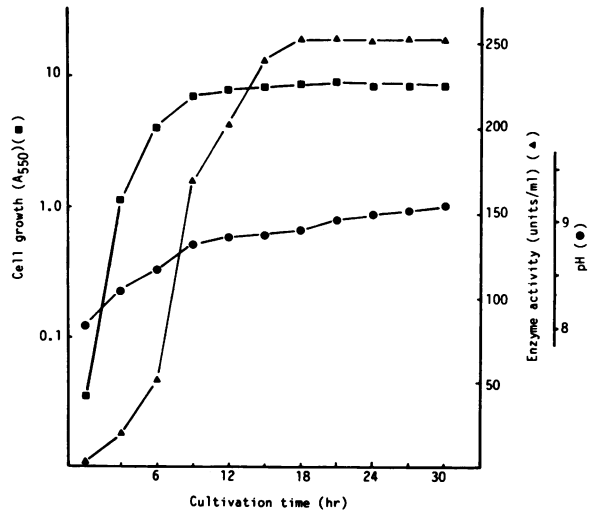

FIG. 4. Growth and D-aminoacylase production in cultures of strain DA181. The culture was carried out as described in the text. Cell growth (■), pH of the broth (●), and the D-amino acylase activity in the toluene-treated cells (▲) were determined.

DISCUSSION

D-Aminoacylase-producing microorganisms were isolated from soil by spreading the soil extract on an isolation

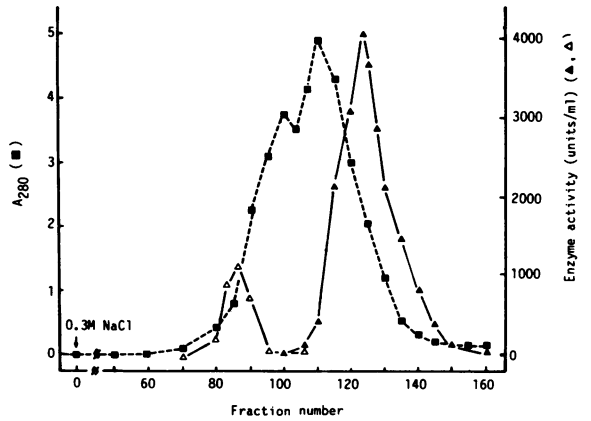

FIG. 5. Purification of D-aminoacylase by DEAE-Sephadex A-50 column chromatography. The dialyzed enzyme preparation was applied after ammonium sulfate precipitation and eluted from the column as described in the text. Fractions of 20 ml were collected. The D-aminoacylase activity (▲) and L-aminoacylase activity (△) and the A_{280} (■) were determined.

TABLE 2. Purification of D-aminoacylase from *A. denitrificans* subsp. *denitrificans* DA181

Purification step	Vol (ml)	Total activity (unit, 10^3)	Total protein (mg)	Sp act (U/mg)	Purification (fold)	Recovery (%)
Crude extract	469	2,196	6,973	315	1	100
$(NH_4)_2SO_4$ precipitation	195	1,691	2,789	606	2	77
DEAE-Sephadex A-50	560	1,243	759	1,638	5	57
Sephadex G-100	400	1,190	177	6,723	21	54
Fractogel TSK DEAE-650	88	827	38.2	21,650	69	38
Sephadex G-100	44	697	16.1	43,290	137	32
Fractogel TSK HW-50	1.1	573	13.1	43,740	139	26
SynChropak AX300	15	239	2.2	108,600	345	11

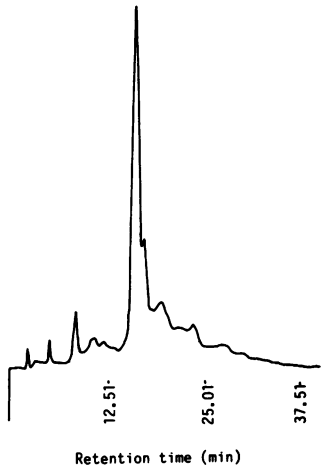

FIG. 6. Purification of D-aminoacylase by SynChropak AX300 high-performance liquid chromatography. The enzyme preparation was applied and eluted from the column as described in the text. The protein that eluted was monitored at 280 nm.

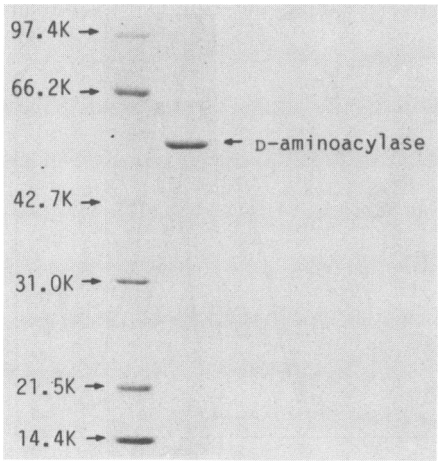

FIG. 7. Sodium dodecyl sulfate-gradient polyacrylamide gel electrophoresis of the purified D-aminoacylase. The gel concentration was a 5 to 20% linear gradient. The molecular weight markers used were phosphorylase *b* (97, 400 [97.4K]), bovine serum albumin (66,200 [66.2K]), ovalbumin (42,700 [42.7K]), carbonic anhydrase (31,000 [31.0K]), soybean trypsin inhibitor (21,500 [21.5K]), and lysozyme (14,400 [14.4K]).

medium containing *N*-acetyl-D-methionine as the sole carbon and nitrogen source. Strains which grew on this medium were considered to possess D-aminoacylase activity. This screening method was very effective for selecting D-aminoacylase-producing microorganisms from soil. The number of microorganisms grown in the isolation medium was 10^2 to 10^3 per g of soil and was 10^7 to 10^8 per g of soil for those grown in a common nutrient broth medium. *A. denitrificans* subsp. *denitrificans* DA181 was selected as the most potent D-aminoacylase producer from about 800 strains that were capable of growing on the isolation medium. When cultivated in LYP medium, strain DA181 produced 250 units of D-aminoacylase per ml of culture broth. It has been reported that *S. olivaceus* (16) and *Pseudomonas* sp. strain 1158 (9) produce 84 and 10 units of D-aminoacylase per ml, respectively. In this study, several other D-aminoacylase-producing strains were also isolated from soil. These strains all belonged to the genus *Alcaligenes*. Yet we failed to detect any of the D-aminoacylase activity in some *Alcaligenes* strains preserved in our laboratory. It appears that the presence of D-aminoacylase activity is not a general characteristic of members of the genus *Alcaligenes*.

The D-aminoacylase of strain DA181 was superior in its stereospecificity and specific activity compared with those of the D-aminoacylases from *S. olivaceus* and *Pseudomonas* strains. This enzyme showed only negligible hydrolysis

TABLE 3. Substrate specificity of D-aminoacylase[a]

Substrate	Relative activity (%) of the[b]:	
	D-Form	L-Form
N-Acetyl-methionine	100	0.1
N-Acetyl-phenylalanine	81	0.3
N-Chloroacetyl-valine	66	0.0
N-Acetyl-leucine	60	0.4
N-Acetyl-alanine	25	0.8
N-Acetyl-tryptophan	33	0.6
N-Acetyl-asparagine	17	0.0
N-Acetyl-alloisoleucine	12	ND[c]
N-Acetyl-valine	6	0.0
N-Acetyl-phenylglycine	5	ND
N-Acetyl-lysine	ND	0.0
N-Acetyl-aspartic acid	ND	0.0
N-Acetyl-tyrosine	ND	0.0
N-Acetyl-arginine	ND	0.0
N-Acetyl-glutamic acid	ND	0.0
N-Acetyl-histidine	ND	0.0

[a] Various substrates (20 mM) were incubated with an appropriate amount of the enzyme for 20 min at 37°C in buffer A. The D- or L-amino acids formed were determined by the TNBS method, as described in the text.

[b] The activity obtained with *N*-acetyl-D-methionine was assigned a value of 100.

[c] ND, Not determined.

activity toward all the *N*-acetyl-L-amino acids tested. The high stereospecificity of this enzyme gives great advantage in the production of D-amino acids. Another merit of this enzyme is its high specific activity, which is 108,600 units per mg, as determined by the TNBS method, with *N*-acetyl-D-methionine used as a substrate. The specific activity of D-aminoacylase from *S. olivaceus* (16) and *Pseudomonas* sp. (9) were 571 and 6.8 units per mg, respectively.

How do the bacteria utilize *N*-acetyl-D-amino acids to support their growth? Members of the genus *Alcaligenes* are chemoorganotrophs that are capable of utilizing a variety of organic acids and amino acids as the sole carbon source. The acetate produced by D-aminoacylase catalyzed hydrolysis of *N*-acetyl-D-amino acids and was considered to be a good carbon source for strain DA181. So far we have not been able to detect any amino acid racemase activity in the crude extract of strain DA181 cells, so it is possible that a transaminase which catalyzes the deamination of D-amino acids might play an important role in the metabolism of D-amino acids produced in the D-aminoacylase reaction.

ACKNOWLEDGMENTS

We express our sincere thanks to S. Mihara and T. Inukai of Amano Pharmaceutical Co. Ltd. (Nagoya, Japan) for kindness in supplying many reagents and helpful discussion and to Fung-Fang Wang for reading the manuscript.

This work was supported by a grant NSC76-0412-B010-19 from the National Science Council, Republic of China.

LITERATURE CITED

1. **Bodansky, M., and D. Perlman.** 1969. Peptide antibiotics. Science **163**:352–358.
2. **Chibata, I., T. Tosa, T. Sato, and T. Mori.** 1976. Production of L-amino acids by aminoacylase adsorbed on DEAE-Sephadex. Methods Enzymol. **44**:746–759.
3. **Cowan, S. T.** 1974. Characters of gram-negative bacteria, p. 77–122. *In* Manual for the identification of medical bacteria, 2nd ed. Cambridge University Press, Cambridge.
4. **Fields, R.** 1972. The rapid determination of amino groups with TNBS. Methods Enzymol. **25**:464–468.
5. **Fukagawa, Y., K. Kubo, T. Ishikura, and K. Kouno.** 1980. Deacetylation of PS-5, a new β-lactam compound. I. Microbial deacetylation of PS-5. J. Antibiot. **33**:543–549.
6. **Kameda, Y., E. Toyoura, and Y. Kimura.** 1958. Occurrence of D-acylase in soil bacteria. Nature (London) **181**:1225.
7. **Kameda, Y., E. Toyoura, H. Yamazoe, Y. Kimura, and Y. Yasuda.** 1952. Hydrolysis and metabolism by soil bacteria of benzoyl derivatives of D-and L-forms of some amino acids. Nature (London) **170**:888–889.
8. **Kersters, K., and J. D. Ley.** 1984. Genus *Alcaligens*, p. 361–373. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
9. **Kubo, K., T. Ishikura, and Y. Fukagawa.** 1980. Deacetylation of PS-5, a new β-lactam compound. II. Separation and purification of L- and D-amino acid acylases from *Pseudomonas* sp. 1158. J. Antibiot. **33**:550–555.
10. **Kubo, K., T. Ishikura, and Y. Fukagawa.** 1980. Deacetylation of PS-5, a new β-lactam compound. III. Enzymological characterization of L-amino acid acylase and D-amino acid acylase from *Pseudomonas* sp. 1158. J. Antibiot. **33**:556–565.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
12. **Lowry, O. H., J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
13. **Sarges, R., and B. Witkop.** 1965. Gramicidin A. VI. The synthesis of valine- and isoleucine-gramicidin A. J. Am. Chem. Soc. **87**:2020–2027.
14. **Sarges, R., and B. Witkop.** 1965. Gramicidin. VII. The structure of valine- and isoleucine-gramicidin B. J. Am. Chem. Soc. **87**:2027–2030.
15. **Sugie, M., and H. Suzuki.** 1978. Purification and properties of D-aminoacylase of *Streptomyces olivaceus*. Agric. Biol. Chem. **42**:107–113.
16. **Sugie, M., and H. Suzuki.** 1980. Optical resolution of DL-amino acids with D-aminoacylase of *Streptomyces*. Agric. Biol. Chem. **44**:1089–1095.
17. **Takahashi, S., T. Ohashi, Y. Kii, H. Kumagai, and H. Yamada.** 1979. Microbial transformation of hydantoins to *N*-carbamyl-D-amino acids. J. Ferment. Technol. **57**:328–332.
18. **Tilton, R. C.** 1981. The genus *Alcaligenes*, p. 856–861. *In* M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes: a handbook on habitats, isolation, and identification of bacteria, vol. 1. Springer-Verlag, New York.
19. **Tosa, T., T. Mori, and I. Chibata.** 1969. Studies on continuous enzyme reactions. Part VI. Enzymatic properties of the DEAE-Sephadex aminoacylase complex. Agric. Biol. Chem. **33**:1053–1059.
20. **Tosa, T., T. Mori, N. Fuse, and I. Chibata.** 1967. Studies on continuous enzyme reactions. IV. Preparation of a DEAE-Sephadex-aminoacylase column and continuous optical resolution of acyl-DL-amino acids. Biotechnol. Bioeng. **9**:603–615.
21. **Tosa, T., T. Mori, N. Fuse, and I. Chibata.** 1969. Studies on continuous enzyme reactions. Part V. Kinetics and industrial application of aminoacylase column for continuous optical resolution of acyl-DL-amino acids. Agric. Biol. Chem. **33**:1047–1052.