APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1986, p. 302–306
0099-2240/86/020302-05$02.00/0

Vol. 51, No. 2

Purification and Characterization of Benzonitrilases from *Arthrobacter* sp. Strain J-1

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Received 13 June 1985/Accepted 4 November 1985

We found two kinds of benzonitrilases, designated benzonitrilases A and B, in a cell extract of *Arthrobacter* sp. strain J-1 grown on benzonitrile as a sole carbon and nitrogen source. Benzonitrilases A and B were purified approximately 409-fold and 38-fold, respectively. Purified benzonitrilase A appeared to be homogeneous according to the criteria of polyacrylamide gel electrophoresis. Both the enzymes hydrolyzed benzonitrile to benzoic acid and ammonia without forming benzamide as an intermediate. The molecular weights of benzonitrilases A and B were found to be 30,000 and 23,000, respectively. The subunit molecular weight of benzonitrilase A was the same as its molecular weight. The isoelectric points of benzonitrilases A and B were 4.95 and 4.80, respectively. The optimum temperature and pH, respectively, for benzonitrilase A were 40°C and 8.5, and those for benzonitrilase B were 30°C and 7.5. The K_m values for benzonitrilases A and B were 6.7 mM and 4.5 mM, respectively. Both the enzymes degraded *p*-tolunitrile, 4-cyanopyridine, and *p*-chlorobenzonitrile, but they did not attack aliphatic nitriles or amides. Both the enzymes were inhibited by thiol reagents.

Despite the widespread use of herbicides containing nitrile groups, such as dichlobenil (2,6-dichlorobenzonitrile), bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), ioxynil (4-hydroxy-3,5-diiodobenzonitrile), and buctril (2,6-dibromo-4-cyanophenyl octanate), comparatively little is known about the microbial metabolism of nitriles and, in particular, the mechanism of cleavage of the C≡N bond by microorganisms. Harper (5, 6) showed that a number of microorganisms isolated from soil were capable of utilizing benzonitrile or 4-hydroxybenzonitrile as a sole carbon and nitrogen source. Recently, we isolated a bacterial strain from a soil sample which could utilize acetonitrile as a sole source of carbon and nitrogen. The strain was classified into the genus *Arthrobacter* and designated *Arthrobacter* sp. strain J-1 (14). Subsequently, it was found that this strain could also grow on benzonitrile as a sole carbon and nitrogen source. In the course of an investigation on the microbial degradation of benzonitrile by this organism, we found two very similar enzymes with only a few differences in their properties. We tentatively designated these two enzymes benzonitrilase A and benzonitrilase B. This is the first report of the presence of two similar benzonitrilases in the same organism. Moreover, the growth of this organism on acetonitrile as a sole carbon and nitrogen source involved an enzyme system which produces only amides, without the corresponding carboxylic acids, from all the substrates tested (2). Thus, this is also the first report from our laboratory of the clarification of two different types of nitrile-degrading enzymes operative in one organism. In this communication the purification and characterization of the two benzonitrilases, A and B, are described.

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MATERIALS AND METHODS

Chemicals. DEAE-Sephacel, Sephadex G-100, and a low-molecular-weight standard kit were obtained from Pharmacia Fine Chemicals. Membrane filters (Diaflo ultrafilter, UM 20) were obtained from Amicon Corp. Ampholytes required for isoelectric focusing were products of LKB-Produktor AB. All other chemicals used were from commercial sources and were of reagent grade.

Microorganism and culture conditions. *Arthrobacter* sp. strain J-1, which can grow on acetonitrile as the sole source of carbon and nitrogen, was used. The basal medium consisted of 13.4 g of K_2HPO_4, 6.5 g of KH_2PO_4, 1 g of NaCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.2 μg of biotin, 0.4 mg of calcium pantothenate, 2 mg of inositol, 0.4 mg of nicotinic acid, 0.4 mg of pyridoxine hydrochloride, 0.4 mg of *p*-aminobenzoic acid, 0.2 mg of riboflavin, and 10 μg of folic acid in 1 liter of tap water. The basal medium supplemented with 0.1% (wt/vol) benzonitrile was used for the stock culture and as the subculture medium. The pH of the medium was adjusted to 7.0 by the addition of 5 M NaOH. *Arthrobacter* sp. strain J-1 was collected from an agar slant and inoculated into the subculture. The subculture (3.5 liters) was shaken reciprocally at 28°C for 48 h and then inoculated into a 100-liter jar fermentor containing 70 liters of the basal medium supplemented with 70 g of benzonitrile and 7 g of antifoam (AF-emulsion; Dow Chemical Co.). Incubation was carried out at 28°C with aeration (30 liters/min), and benzonitrile (70 g) was fed after 24, 32, and 38 h of cultivation. After 45 h of cultivation, the cells were harvested by continuous-flow centrifugation and then washed twice with 0.15 M NaCl. The yield of wet cells was approximately 0.96 g/liter of medium. In some cases, the washed cells were stored at −80°C as a frozen paste until used.

Enzyme assay and protein determination. Benzonitrilase activity was monitored by routinely measuring the formation of ammonia from benzonitrile by the indophenol method

(phenol-hypochlorite method) (4). The standard reaction mixture consisted of 50 μmol of potassium phosphate buffer (pH 8.0), 3 μmol of benzonitrile, and an appropriate amount of enzyme in a total volume of 0.5 ml. The reaction was started by adding the substrate and was carried out at 30°C for various times, depending on the experiment. The reaction for both enzymes was not linear with respect to time, especially in the early phase of the reaction, so the activity was always determined from the linear portion of the time course plot, and a quantitative assessment of the lag period was always performed by extrapolating the linear portion of the time axis.

One unit of enzyme activity was defined as the amount of enzyme which catalyzed the formation of 1 μmol of ammonia per min.

Protein was determined by the method of Lowry et al. (9) with bovine serum albumin as a standard or from the A_{280} by using an E value of 10.0 for 10 mg/ml and a 1-cm light path.

Preparation of amidase. Amidase was prepared from acetonitrile-grown *Arthrobacter* sp. strain J-1 cells as described previously (3).

Other assays. Various nitriles, amides, and acids were determined by gas-liquid chromatography. The reaction was terminated by the addition of 50 μl of 1 N HCl, and then a portion of the reaction mixture was applied to a Shimadzu model GC-4CM gas-liquid chromatograph equipped with a flame ionization detector. A glass column (3 mm [inside diameter] by 1 m) packed with Porapak Q (80/100 mesh) was used, and the injection and detector temperatures were 240 and 210°C, respectively. The carrier gas was N_2 at 40 cm^3/min. Integration and calibration of peaks were carried out with a Shimadzu Chromatopack C-R1A.

Polyacrylamide gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis was performed in 10% polyacrylamide slab gels with the Tris-glycine buffer system described by King and Laemmli (8). The enzyme (40 μg) was subjected to electrophoresis in the presence of 0.1% sodium dodecyl sulfate at a current of 30 mA per slab. The gels were stained for protein with Coomassie brilliant blue G-250 and destained in ethanol-acetic acid-H_2O (3:1:6). The relative molecular mass of the enzyme subunit was determined by comparison with the relative mobilities of the standard proteins phosphorylase *b* (M_r, 94,000), bovine serum albumin (M_r, 67,000), ovalbumin (M_r, 43,000), bovine erythrocyte carbon anhydrase (M_r, 30,000), and soybean trypsin inhibitor (M_r, 20,100).

Isoelectric focusing. The isoelectric point of the enzyme was determined as described by Winter and Karlson (13). The density gradient with a pH range of 3 to 10 contained 2% ampholyte. A sample of the enzyme (about 1.0 mg) which had been exhaustively dialyzed against 0.13 M glycine was applied to the column after about half of the sucrose gradient had been formed. Electrofocusing was carried out at 5°C until there were no further changes in the current (72 h), during which time the voltage was increased from 300 to 600 V. The column was then attached to a fraction collector, and 1.5-ml fractions were collected until the column had been emptied. The A_{280}, the pH, and the benzonitrilase activity of the fractions were measured.

Gel filtration on calibrated Sephadex G-100. Analytical gel chromatography of the enzyme was performed on a column (3.0 by 120 cm) packed with Sephadex G-100. The gel bed was equilibrated with 0.01 M potassium phosphate buffer (pH 7.0) containing 0.2 M KCl at 5°C. A constant flow of the equilibration buffer was maintained with a peristaltic pump (type-2132; LKB-Produktor AB). Small samples (1.0 ml) were chromatographed in all the experiments. The molecular weight of the enzyme was estimated by the method of Andrews (1). Standard proteins (Combithek) for the calibration were products of Boehringer GmbH.

RESULTS AND DISCUSSION

Time course of cultivation of *Arthrobacter* sp. strain J-1 on benzonitrile. The time course of growth of the strain on benzonitrile is shown in Fig. 1. Exponential growth began after 6 h of cultivation. As benzonitrile was degraded, benzoic acid and ammonia were successively formed and accumulated in the culture broth. Benzamide was not detected throughout the cultivation at concentrations higher than 50 μM, the lowest detection limit of gas-liquid chromatography under the conditions we used.

Effect of carbon and nitrogen sources on the formation of benzonitrilase. *Arthrobacter* sp. strain J-1 was cultivated in basal medium (1 liter). After 2 days of cultivation at 28°C, washed cells (33 mg) were prepared and inoculated into the basal medium to which benzonitrile (1 g), *n*-butyronitrile (3.91 g), acetonitrile (3.85 g), sodium acetate (5 g), sodium succinate (5 g), glucose (5 g), or ammonium sulfate (2 g) had been added, and then cultured at 28°C for various times (Table 1). The cells were harvested by centrifugation and disrupted with an ultrasonic oscillator in 0.1 M potassium phosphate buffer (pH 7.0) containing 1 mM 2-mercaptoethanol and 1 mM EDTA. The cell debris was removed by

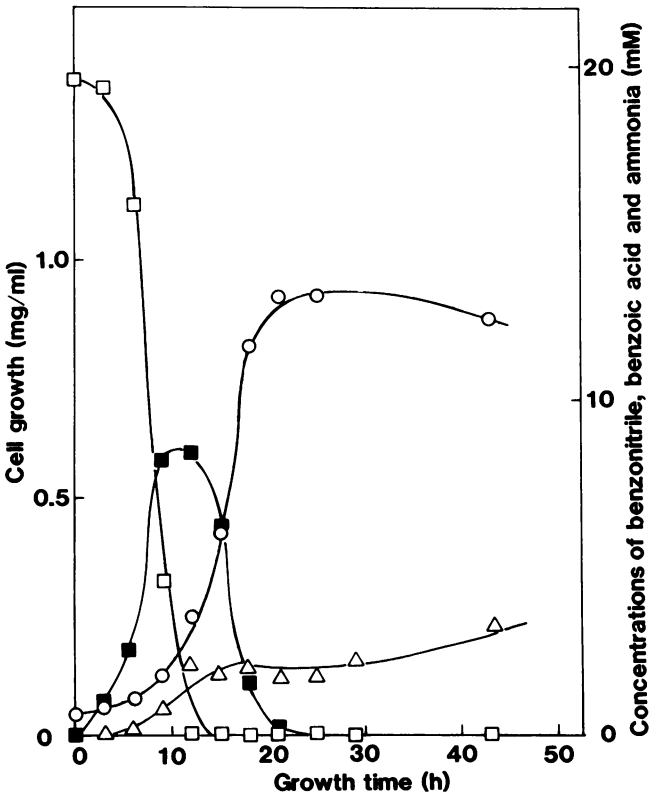

FIG. 1. Substrate disappearance and product formation during the growth of *Arthrobacter* sp. strain J-1. Cultivation was carried out at 28°C in a 2-liter flask containing 500 ml of the basal medium supplemented with 10 mM benzonitrile with reciprocal shaking. The bacterium was grown on benzonitrile (□); cell growth (○) and the formation of benzoic acid (■) and ammonia (△) were measured.

TABLE 1. Effect of carbon and nitrogen sources on the formation of benzonitrilase in *Arthrobacter* sp. strain J-1

Carbon and nitrogen sources	Cultivation time (h)	Sp act (U [10^{-3}]/mg) of:	
		Benzonitrilase	Benzamidase
Benzonitrile	24	162	ND[a]
	48	260	ND
n-Butyronitrile	36	640	ND
	48	177	ND
Acetonitrile	24	70	ND
Sodium acetate + ammonium sulfate	48	1.0	ND
Sodium succinate + ammonium sulfate	24	2.3	ND
Glucose + ammonium sulfate	48	2.0	ND

[a] ND, Not detected.

centrifugation at 12,000 × *g* for 30 min, and then benzonitrilase and benzamidase in the supernatant solution were assayed. Benzonitrilase was inducibly formed when the strain was grown on benzonitrile, *n*-butyronitrile, or acetonitrile (Table 1). The amidase activity of the hydrolysis of benzamide was not detected with all growth substrates tested.

Purification of benzonitrilases A and B. All purification steps were performed at 0 to 5°C. The enzymes were highly unstable; after several trials, the enzymes could be stabilized to some extent in 0.01 to 0.1 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.0) containing 20% (wt/vol) glycerol, 1 mM 2-mercaptoethanol, and 1 mM EDTA. This buffer solution was used throughout the purification steps unless otherwise specified.

(i) Step 1: preparation of a cell-free extract. Washed cells (9.6 g) harvested from 10 liters of the culture medium were suspended in 0.1 M HEPES buffer (pH 7.0) and disrupted with a Dyno-mill (W. A. Bachofen) for 1 h. The resulting solution was centrifuged at 15,000 × *g* for 30 min, and the supernatant solution was dialyzed overnight against 0.01 M HEPES buffer (pH 7.0).

(ii) Step 2: ultracentrifugation. The dialysate was centrifuged at 100,000 × *g* for 2 h at 4°C in a 50.3 Ti rotor with a model L5B Beckman ultracentrifuge. Most of the activity was found in the supernatant solution.

(iii) Step 3: first DEAE-Sephacel column chromatography. The above enzyme solution was placed on a DEAE-Sephacel column (5 by 30 cm) equilibrated with 0.01 M HEPES buffer (pH 7.0). After the column was washed with the same buffer, enzyme elution was performed in a stepwise manner with 0.01 M HEPES buffer (pH 7.0) containing 0.1 M, 0.2 M, and 0.3 M KCl. The enzyme was eluted in two peaks with 0.2 M and 0.3 M KCl. The two peaks of benzonitrilase activity (A and B in the order of elution) were individually pooled.

(iv) Step 4: second DEAE-Sephacel column chromatography. The pooled benzonitrilase A from the previous step was applied to a second DEAE-Sephacel column (2.5 by 25 cm) equilibrated with 0.01 M HEPES buffer (pH 7.8), and enzyme elution was performed with a linear gradient of 0 to 0.5 M KCl in the same buffer. The most active fractions were combined (235 ml) and concentrated to about 2 ml with an Amicon filtration unit.

Similarly, the pooled benzonitrilase B from the previous step was applied to another DEAE-Sephacel column (3 by 33 cm) equilibrated with 0.01 M HEPES buffer (pH 7.8), and enzyme elution was performed with a linear gradient of 0 to 0.5 M KCl in the same buffer. The most active fractions of the enzyme were pooled (180 ml) and concentrated to about 2 ml with the Amicon filtration unit.

(v) Step 5: Sephadex G-100 column chromatography. The concentrated enzyme solutions (benzonitrilases A and B) from the previous step were placed on a Sephadex G-100 column (1.0 by 80 cm) separately and repeatedly eluted with 0.01 M HEPES buffer (pH 7.0). The elution position of the protein coincided with the position of the enzyme activity in the case of benzonitrilase A.

Approximately 409-fold purification of benzonitrilase A was achieved, with a yield of 35% (Table 2). Purified benzonitrilase A catalyzed the hydrolysis of benzonitrile, at 1.31 μmol/min per mg of protein, under standard conditions. Benzonitrilase B was purified only 37.9-fold, with a yield of 11% (Table 2). Purified benzonitrilase B catalyzed the hydrolysis of benzonitrile, at 0.122 μmol/min per mg of protein, under standard conditions.

Homogeneity. The homogeneity of benzonitrilases A and B was investigated by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. Benzonitrilase A was found to be homogeneous and appeared as a single band. However, benzonitrilase B could not be purified to homogeneity.

Molecular weight and isoelectric point. The molecular weights of benzonitrilases A and B were estimated to be 30,000 and 23,000, respectively, on a calibrated column of Sephadex G-100. The subunit molecular weight of benzonitrilase A was determined to be 30,000, according to its relative mobility in sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. This implies that benzonitrilase A exists as a monomer. The purified benzonitrilase B sample appeared as a main band and several minor bands on the slab gel.

The isoelectric points of benzonitrilases A and B were determined separately with 2% ampholyte (pH 3.5 to 10) and were found to be 4.95 and 4.80, respectively. In a separate experiment, the two enzymes were mixed and subjected

TABLE 2. Purification of benzonitrilases A and B from *Arthrobacter* sp. strain J-1

Step	Benzonitrilase A				Benzonitrilase B			
	Total protein (mg)	Total activity (U)	Sp act (U/mg)	% Recovery	Total protein (mg)	Total activity (U)	Sp act (U/mg)	% Recovery
1	81,900	264	0.0032	100				
2	41,300	256	0.0062	97				
3	1,380	180	0.141	68	1,150	104	0.090	39
4	453	162	0.351	61	720	74.2	0.103	28
5	70	91.8	1.31	35	245	29.8	0.122	11

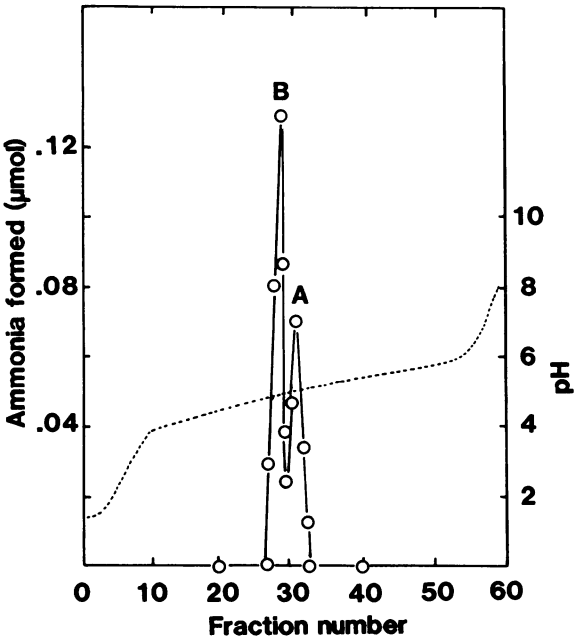

FIG. 2. Isoelectric focusing of benzonitrilases A and B. Both the enzymes (benzonitrilase A, 0.27 U; benzonitrilase B, 0.146 U) were applied to the same sucrose density gradient as described in Materials and Methods. (○) Benzonitrilase activity; -----, pH of the eluate.

together to isoelectric focusing with 2% ampholyte (pH 4.0 to 6.0). Benzonitrilases A and B appeared as separate peaks (Fig. 2). having isoelectric points of 4.95 and 4.80, respectively, as was found in the previous experiment.

Effect of enzyme concentration. The effect of the enzyme concentration on the time course of the reaction was examined. The reaction was not linear with respect to time, especially in the early phase of the reaction and at low enzyme concentrations. On incubation with a substrate, benzonitrilase A showed time-dependent activation. Neither benzoic acid nor ammonia had any effect on the length of the lag period. This phenomenon was also observed with benzonitrilase B.

Substrate specificity. The substrate specificities of the two enzymes are shown in Table 3. Both benzonitrilases A and B were significantly active towards benzonitrile, 4-cyanopyridine, *p*-tolunitrile, and *p*-chlorobenzonitrile. However, the enzymes were inactive towards *p*-cyanobenzoic acid, *p*-nitrobenzonitrile, 2-methylglutaronitrile, *n*-capronitrile, glutaronitrile, crotononitrile, methacrylonitrile, *n*-valeronitrile, adiponitrile, lactonitrile, chloroacetonitrile, isobutyronitrile, methoxyacetonitrile, and hydroxyacetonitrile under the same conditions. The nitrile hydratase activity of both enzymes was examined by adding 0.01 U of either benzonitrilase A or B and 0.01 U of amidase prepared from acetonitrile-grown *Arthrobacter* sp. strain J-1 cells; however, neither enzyme catalyzed the formation of benzamide, benzylamide, acetamide, acrylamide, propionamide, or *n*-butyramide from benzonitrile, benzylnitrile, acetonitrile, acrylonitrile, propionitrile, or *n*-butyronitrile, respectively. Neither enzyme exhibited amidase activity with phenylacetamide, *n*-butyramide, isobutyramide, methacrylamide, crotonamide, α-cyanoacetamide, malonamide, α-chloroacetamide, lactamide, succinamide, iodoacetamide, formamide, propionamide, *n*-valeramide, acrylamide, acetamide, or benzamide, even with the addition of excess enzyme (0.02 U).

The K_m values for benzonitriles A and B were 6.7 mM and 4.5 mM, respectively.

Stoichiometry. A time course study of benzonitrile hydrolysis was carried out. For each mole of benzonitrile hydrolyzed by benzonitrilases A and B, 1 mol of benzoate and 1 mol of ammonia were formed.

Effect of temperature on the activity and stability of the enzymes. Benzonitrilase A showed maximum activity at 40°C, whereas the optimum temperature for the activity of benzonitrilase B was 30°C. Benzonitrilase A was stable up to about 45°C for 20 min, and then the activity fell rapidly above that temperature; bezonitrilase B was gradually degraded above 30°C.

Effect of pH on the activity of the enzymes. The optimum pHs for the activity of benzonitrilases A and B in the presence of 0.1 M potassium phosphate buffer or 0.1 M HEPES buffer were 8.5 and 7.5, respectively. However, in 0.1 M sodium borate buffer, benzonitrilase A was found to be completely inactive, whereas benzonitrilase B showed about 64% activity at pH 7.5 relative to that in potassium phosphate buffer.

Effects of various reagents and metal ions on the activity of the enzymes. The enzymes were preincubated for 30 min at 30°C with 0.1 mM concentrations of various reagents. The sensitivity to Cu^{2+} was different for the two enzymes. Benzonitrilase A was inhibited 30% with Cu^{2+}, whereas the inhibition of benzonitrilase B was 83%. Both the enzymes were found to be very sensitive to *p*-chloromercuribenzoic acid, Hg^{2+}, and Ag^{+} (91 to 100% inhibition). This indicates that thiol groups play an important role in the activity of the enzymes. Azide, cyanide, EDTA, Co^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Mn^{2+}, and Pb^{+} did not affect the activity of either of the enzymes.

Effect of salts on the activity of the enzymes. The enzyme activities were measured in the presence of KCl, NaCl, $MgCl_2$, and $CaCl_2$ at final concentrations of 1 mM, 10 mM, and 50 mM. $CaCl_2$ inhibited both the enzymes at the final concentration of 50 mM. The other salts had no effect on the activity of the enzymes. High concentrations of salts could not abolish the lag phase of the enzyme reaction.

Harper (5–7) showed that in *Nocardia rhodochrous*, benzonitrile was directly hydrolyzed to benzoic acid and ammonia by nitrilase. *Arthrobacter* sp. strain J-1 decomposes benzonitrile in the same way, as it grows on benzonitrile as a sole source of carbon and nitrogen. However, *Arthrobacter* sp. strain J-1 produces two very similar benzonitrilases (A and B) with some differences in their properties. Benzonitrilases A and B seem to be isozymes. At this stage it is not clear how these two enzymes participate in the degradation of benzonitrile.

TABLE 3. Substrate specificities of benzonitrilases A and B[a]

Substrate	% Relative activity of:	
	Benzonitrilase A	Benzonitrilase B
Benzonitrile	100	100
p-Tolunitrile	125	112
4-Cyanopyridine	44	79
p-Chlorobenzonitrile	71	96

[a] Various nitriles (3 μmol) were incubated for 10 min at 30°C in reaction mixtures (0.5 ml) containing 0.01 U of benzonitrilase A or B and 50 μmol of potassium phosphate buffer (pH 8.0). The rate of ammonia formation was determined.

It is interesting to note that when this organism was grown on acetonitrile as a sole carbon and nitrogen source, it produced aliphatic nitrile hydratase (2) and amidase (13). Nitrile hydratase transforms aliphatic nitriles to the corresponding amides only, without the formation of carboxylic acids and ammonia. Therefore, there are at least two distinct pathways for nitrile hydrolysis in this microorganism. One is a pathway in which both nitrile hydratase and amidase are involved, and the other is one in which only nitrilase is involved. The chemical hydrolysis of acetonitrile, propionitrile, and benzonitrile proceeds exclusively via amides (10–12). Whether there is any correlation between the kind of nitrile and the kind of hydrolytic enzyme would be of interest in enzymology. It is possible that nitriles with saturated alkyl groups, such as acetonitrile, are hydrated by nitrile hydratase, followed by hydrolysis of the amides by amidase. On the other hand, benzonitrile and acrylonitrile (unpublished data) are degraded directly to carboxylic acids and ammonia. It is possible that nitriles are directly hydrolyzed to carboxylic acids and ammonia if the cyanogen group is conjugated with a double bond, although further studies are required.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

LITERATURE CITED

1. **Andrews, P.** 1965. The gel-filtration behaviour of proteins related to their molecular weights over a wide range. Biochem. J. **96**:596–606.
2. **Asano, Y., K. Fujishiro, Y. Tani, and H. Yamada.** 1982. Aliphatic nitrile hydratase from *Arthrobacter* sp. J-1: purification and characterization. Agric. Biol. Chem. **46**:1165–1174.
3. **Asano, Y., M. Tachibana, Y. Tani, and H. Yamada.** 1982. Purification and characterization of amidase which participates in nitrile degradation. Agric. Biol. Chem. **46**:1175–1181.
4. **Fawcett, J. K., and J. E. Scott.** 1960. A rapid and precise method for the determination of urea. J. Clin. Pathol. **13**:156–159.
5. **Harper, D. B.** 1976. Purification and properties of an unusual nitrilase from *Nocardia* N.C.I.B. 11216. Biochem. Soc. Trans. **4**:502–504.
6. **Harper, D. B.** 1977. Microbial metabolism of aromatic nitriles. Biochem. J. **165**:309–319.
7. **Harper, D. B.** 1977. Fungal degradation of aromatic nitriles. Biochem. J. **167**:685–692.
8. **King, J., and U. K. Laemmli.** 1971. Polypeptides of the tail fibers of bacteriophage T4. J. Mol. Biol. **62**:465–477.
9. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
10. **Rabinovitch, B. S., and C. A. Winkler.** 1942. The hydrolysis of aliphatic nitriles in concentrated hydrochloric acid solutions. Cand. J. Res. Sect. B **20**:221–230.
11. **Rabinovitch, B. S., C. A. Winkler, and A. R. P. Stewart.** 1942. The hydrolysis of propionitrile in concentrated hydrochloric acid solutions. Can. J. Res. Sect. B **20**:121–132.
12. **Rabinovitch, B. S., C. A. Winkler, and A. R. P. Stewart.** 1942. Kinetics of the alkaline hydrolysis of propionitrile. Cand. J. Res. Sect. B **20**:185–188.
13. **Winter, A., and C. Karlson.** 1976. LKB application note 219. LKB Produktor AB, Bromma, Sweden.
14. **Yamada, H., Y. Asano, T. Hino, and Y. Tani.** 1979. Microbial utilization of acrylonitrile. J. Ferment. Technol. **57**:8–14.