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Enantioselective hydrolysis of O-acetylmandelonitrile to O-acetylmandelic acid by bacterial nitrilases

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Abstract. Bacteria were enriched from soil samples, using benzylcyanide, α-methyl-, α-ethyl- or α-methoxybenzylcyanide as the sole source of nitrogen. All isolated strains belonged to the genus *Pseudomonas*. Resting cells of the isolates hydrolysed O-acetylmandelonitrile to O-acetylmandelic acid, O-acetylmandelic acid amide and mandelic acid. From racemic O-acetylmandelonitrile all isolates preferentially formed R(−)-acetylmandelic acid (= D-acetylmandelic acid). The enantioselective hydrolysis of O-acetylmandelonitrile could also be demonstrated in vitro. Crude extracts did not hydrolyse O-acetylmandelic acid amide indicating an enantioselective nitrilase rather than a nitrile hydratase/amidase system.

Key words: Nitriles – Enantioselective enzymes – *Pseudomonas* – Mandelic acid derivatives

α-Hydroxycarboxylic acids are potential precursors for different chemical processes, e.g. the synthesis of antibiotics (Jallageas et al. 1980). Various bacterial and fungal systems with the ability to hydrolyse nitriles either directly or via the corresponding amides have been described (Harper 1977a, b; Nagasawa et al. 1987, 1988a, b, 1990; Watanabe et al. 1987). Yamamoto et al. (1991) produced R(−)-mandelic acid from racemic mandelonitrile by using an enantioselective nitrilase from *Alcaligenes faecalis* ATCC 8750.

In the present publication the potential of bacterial enzymes for the synthesis of aromatic, optically active α-hydroxycarboxylic acids was evaluated. Enantioselective synthesis of optically active mandelic acid derivatives was achieved.

A preliminary account of this work was presented previously (N. Layh et al. 1991).

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Materials and methods

Chemicals

O-Acetylmandelic acid was prepared by acetylation of mandelic acid (La Mer and Greenspan 1934). R- and S- O-acetylmandelic acids were obtained by the same procedure from R- or S-mandelic acid. O-Acetylmandelic acid amide was synthesized following the procedure described by Anschütz and Böcker (1909). The method of Utimoto et al. (1981) was used for the synthesis of methoxybenzylcyanide. O-Acetylmandelonitrile was prepared from mandelonitrile and acetyl chloride in pyridine (Effenberger et al. 1991).

Benzylcyanide, methylbenzylcyanide and ethylbenzylcyanide were purchased from Aldrich (Steinheim, FRG). D- and L-mandelic acid were obtained from Sigma (Deisenhofen, FRG).

Nutrient Broth was obtained from Difco (Detroit, Mich., USA). All other chemicals used for mineral salts media and buffer solutions were purchased from Aldrich, E. Merck AG (Darmstadt, FRG) or Fluka (Buchs, Switzerland).

Media

Nitrogen-free media were prepared in a mineral medium containing per liter: 14 g $Na_2HPO_4 \cdot 12\,H_2O$, 2 g KH_2PO_4, 0.05 g $CaCl_2 \cdot 2\,H_2O$, 0.05 g Fe^{3+}-citrate, 0.02 g $MgSO_4 \cdot 7\,H_2O$, and 1 ml of a trace element solution described by Pfennig and Lippert (1966) without iron salts and EDTA.

Solid media were prepared by adding 15 g of Agar No. 1 (Oxoid LTD, Basingstoke, Hampshire UK) to each litre of mineral medium containing the appropriate nitrogen and carbon source.

Screening and isolation of microorganisms

Bacterial cultures were isolated by enrichment in cultures using Erlenmeyer flasks with baffles (500 ml with 50 ml of medium). Cultures were incubated at 30 °C on a rotary shaker at 120 rpm.

Enrichments were started with different soils taken from the area of Stuttgart (Germany) and carried out in a nitrogen free mineral salts medium containing succinate (10 mM) and benzylcyanide, α-methyl-, α-ethyl-, or α-methoxybenzylcyanide (0.5 mM each), respectively, as a nitrogen source. Growth of eukaryotic cells was suppressed by repeated exposure to cycloheximide (0.1% w/v). The cultures were transferred weekly to fresh medium (1:5 v/v). After one month the enrichment cultures were transferred to agar plates with succinate and the respective nitriles as sole nitrogen source. Nitrogen fixing clones were detected by their growth in the absence of nitriles.

Initial characterization and taxonomic classification of the isolates

On Nutrient Broth agar all isolates initially formed round, transparent colonies, which reached a diameter of 3–5 mm after six days at 30 °C. After prolonged incubation the colonies of *Pseudomonas putida* BC10 and *P. fluorescens* MOBC3 became irregular. Colonies from *P. fluorescens* EBC191 were mucoid. The gram-negative isolates were characterized by using the API 20 NE test system (API-System S.A., La Balme les Grottes, France). The results thus obtained were interpreted using the "APILAB PC (Version 2.0)"-software. Thus strain BC10 was found to belong to *Pseudomonas putida* (99.5% probability); strains MBC1 (99.8%), EBC 191 (99.9%) and MOBC3 (98.2%) were identified as *Pseudomonas fluorescens*.

Measurement of growth

Growth of bacterial cultures was either monitored spectrophotometrically by measuring the optical density at 546 nm with a Kontron Uvikon 820 Spectrophotometer (Kontron, Eching, FRG) or with a Klett-Summerson Colorimeter (Modell 800-3; Klett MFG Co. Inc., New York, USA). Klett Units were converted into optical density, whereas 100 Klett Units corresponded to an optical density $OD_{546\,nm}$ of about 0.6.

Growth of bacteria and turnover of nitriles by resting cells

To obtain cells with high nitrile hydrolysing activity the bacteria were grown in media with succinate (10 mM) and the respective nitrile (conc. = 0.5–1 mM; usually benzylcyanide) as sole nitrogen source. Bacteria were harvested by centrifugation (6500 × g, 4 °C, 20 min) and resuspended in Na/K-phosphate buffer (54 mM, pH 7.4) to a final optical density ($OD_{546\,nm}$) of 10. These cell-suspensions were incubated on a rotary shaker (30 °C, 120 rpm) and nitriles (or amides, carboxylic acids) added.

Analytical methods

O-Acetylmandelonitrile and its hydrolysis products were analyzed by high pressure liquid chromatography (HPLC). The system consisted of a pump (Liquid Chromatograph 655A-11; Merck, Darmstadt, FRG) equipped with a detector (Spectroflow 783; Krátos, Karlsruhe, FRG) and a chromatointegrator (D 2000; Merck).

If no detection of enantiomers was necessary a reverse-phase column (125 mm by 4.6 mm [internal diameter]; Bischoff, Leonberg, FRG and LiChrosorb RP8 with 5 µm particles [Merck]) was used to separate individual compounds which were detected spectrophotometrically at 210 nm. For separation of enantiomers a Chiralcel OD column (28 cm length; Baker, Deventer, The Netherlands) was used. The mobile phases used and retention times are given in Table 1.

Preparation of samples for HPLC analysis using the Chiralcel OD column

According to the manufacturer the samples to be analyzed had to be dissolved in a water-free organic solvent. Therefore the aqueous solutions (0.6 ml each) obtained from biological conversions were acidified with 50 µl 1M HCl and ethylacetate was added to a final volume of 1.25 ml. The phases were thoroughly mixed and then separated by a short centrifugation (10 s, 11000 × g). The organic phase was then analyzed by HPLC.

Preparation of cell-free extracts

Cell-suspensions in 54 mM Na/K-phosphate buffer pH 7.4 were disrupted by using a French press (Aminco, Silver Springs, Md. USA) at 80 MPa. Cell debris was removed by centrifugation at 100000 × g for 30 min at 4 °C. Protein was determined by the method of Bradford (1976), using bovine serum albumin as a standard.

Table 1. Solvent systems as mobile phases for HPLC of O-acetylmandelonitrile, its hydrolysis products and other nitriles

Nitrile or product	Column[a]	Mobile phase[b]	Retention time[c] (min)
AMA	RP8	A	4.9
AMAA	RP8	A	2.9
AMN	RP8	A	7.8
MA	RP8	A	2.4
MN	RP8	A	3.5
BC	RP8	A	4.9
MBC	RP8	A	7.9
MOBC	RP8	A	7.0
EBC	RP8	B	3.2
S(+)-AMA	Chira	C	7.0
R(−)-AMA	Chira	C	7.6
S(+)-AMN	Chira	C	7.4
R(−)-AMN	Chira	C	8.0
S(+)-MA	Chira	C	10.0
R(−)-MA	Chira	C	11.8

[a] RP8 LiChrosorb RP8 (Merck, Darmstadt, FRG), Chira Chiralcel OD (Baker, Deventer, The Netherlands)
[b] A water containing 45% (v/v) methanol and 0.3% (v/v) H_3PO_4; B water containing 70% (v/v) methanol and 0.3% (v/v) H_3PO_4; c hexane containing 10% (v/v) isopropanol and 0.1% (v/v) H_3PO_4
[c] Retention times measure a flow rate of 1 ml/min
Abbreviations: AMA O-acetylmandelic acid; AMAA O-acetylmandelic acid amide; AMN O-acetylmandelonitrile; BC Benzylcyanide; EBC α-ethylbenzylcyanide; MA mandelic acid; MBC α-methylbenzylcyanide; MN mandelonitrile; MOBC α-methoxybenzylcyanide

Enzyme assays

Nitrilase activity was assayed in a reaction mixture (1 ml) containing 54 µmol potassium phosphate pH 7.4, 1 µmol nitrile and 0.5 mg protein (cell-free extract). The reaction was started by adding the substrate and was carried out at 30 °C. After various times the reaction was stopped by adding 100 µl reaction mixture onto 10 µl 1 M HCl. One unit of enzyme activity was defined as the amount of enzyme that converts 1 µmol of substrate per minute.

Results

Enrichment of bacteria

To obtain microorganisms with the ability to hydrolyse aromatic nitriles these compounds were supplied as sole nitrogen source, while succinate served as a carbon source. Mandelonitrile could not be used as a substrate for enrichment, because at pH 7 it spontaneously hydrolyses to benzaldehyde and HCN. Thus enrichments with mandelonitrile as nitrogen source would result in bacteria able to use HCN as a nitrogen source. O-Acetylmandelonitrile, although being stable in aqueous solutions at pH 7, could also not be used as a substrate for enrichment, because ubiquitous bacterial esterases would again generate mandelonitrile. Therefore structural analogues of O-acetylmandelonitrile, which carried no hydrolysable group except the nitrile function, were

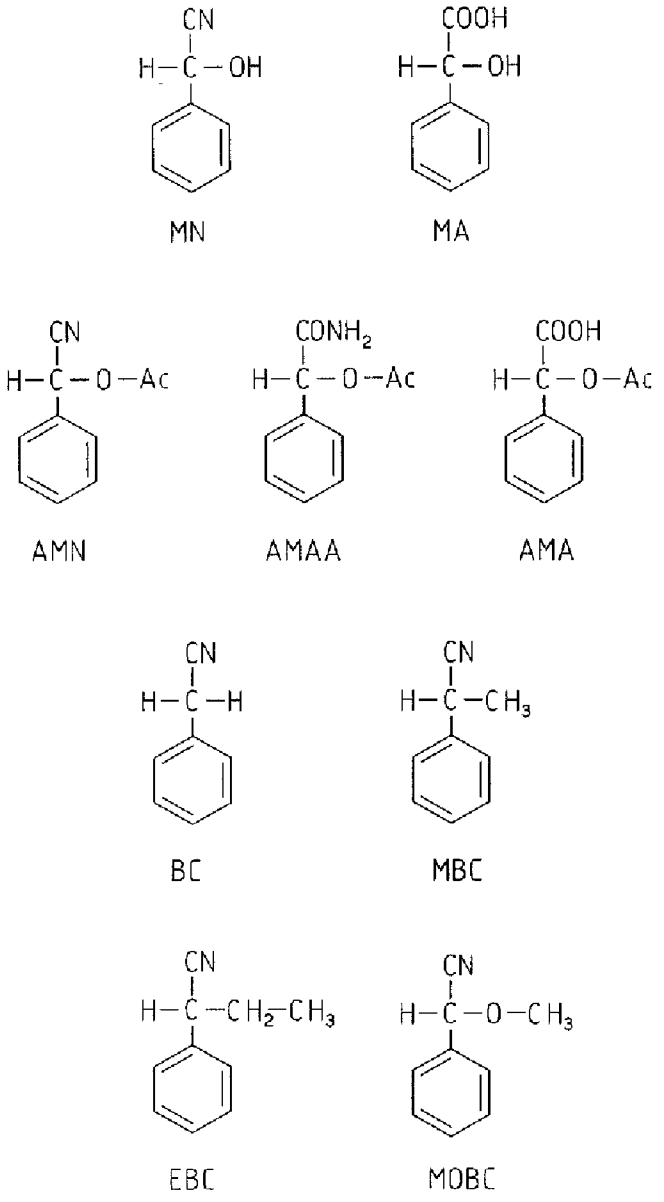

Fig. 1. Structural formulas of different compounds used during this study. AMA O-acetylmandelic acid; AMAA O-acetylmandelic acid amide; AMN O-acetylmandelonitrile; BC benzylcyanide; EBC α-ethylbenzylcyanide; MA mandelic acid; MBC: α-methylbenzylcyanide; MN: mandelonitrile; MOBC: α-methoxybenzylcyanide

used as enrichment substrates. Thus enrichments were performed with benzylcyanide, α-methylbenzylcyanide, α-ethylbenzylcyanide or α-methoxybenzylcyanide as sole nitrogen source (Fig. 1).

Isolation of microorganisms

From each enrichment culture containing different substrate analogues as a N-source one fastgrowing strain was choosen for further studies. All isolates were Gram-negative (Scherrer 1984; Gregersen 1978) motile rods. The species were tentatively determined (see 'Material and methods') and the strains designated according to their respective enrichment substrate as *Pseudomonas putida* BC10, *Pseudomonas fluorescens* EBC191, MBC1 and MOBC3. To ensure that the isolates belonging to the species *Pseudomonas fluorescens* were really different isolates, growth with various carbon sources was compared. As demonstrated by Table 2 it is evident that indeed a wide range of physiological different strains had been enriched.

Compared with succinate all strains grew faster with glucose, fructose or trehalose. Therefore the following experiments were performed with glucose as source of carbon and energy.

Nitriles as nitrogen sources

Different aliphatic and aromatic amides or nitriles were tested as potential nitrogen sources for the isolates. Regardless of the nitrogen source used as an enrichment substrate all strains utilized benzylcyanide, α-methylbenzylcyanide and α-methoxybenzylcyanide (Table 3). In addition the *Pseudomonas fluorescens* strains could utilize α-ethylbenzylcyanide (3 mM) as a nitrogen source. At concentrations lower than 1 mM α-ethylbenzylcyanide also served as nitrogen source for *Pseudomonas putida* BC10. Only few aliphatic amides and nitriles were utilized as N-source (Table 3).

Transformation of O-acetylmandelonitrile

Resting cells of all newly isolated strains were able to convert O-acetylmandelonitrile to O-acetylmandelic acid. In addition, varying amounts of O-acetylmandelic acid amide, mandelic acid and benzoate were excreted into the medium by certain isolates. The formation of O-acetylmandelic acid and O-acetylmandelic acid amide

Table 2. Growth of the newly isolated bacteria with various carbon sources

Growth substrate	Strains[a]			
	BC10	MBC1	MOBC3	EBC191
Acetamide	–	–	+	–
D-Cellobiose	–	–	+	–
Glycolate	+	–	M	–
D-Maltose	–	–	+	+
Mannitol	(+)	+	+	+
D,L-Norleucine	–	(+)	+	–
Starch	–	–	+	–
Testosterone	–	–	+	–
D-Tryptophane	–	–	+	–
4-Hydroxybenzoate	+	+	+	–

+ growth; – no growth; (+) weak growth; M few "mutant" colonies

[a] BC10 *Pseudomonas putida* BC10; MBC1, MOBC3 and EBC191 *Pseudomonas fluorescens* MBC1, MOBC3 and EBC191

All strains grew with acetate, D,L-arginine, betaine, fructose, glucose, *myo*-inositol, D,L-lactate, malate, succinate, trehalose, valine, benzoate, mandelate or salicylate

None of the strains grew with adipate, fucose, geraniol, maleate. The isolates were incubated on agar-plates with the respective substrate (3 mM each) as sole source of carbon and energy. Growth was stated after 15 days incubation at 30 °C

Table 3. Utilization of various amides and nitriles as nitrogen sources for growth of the isolates

Amide/nitrile	Strains[a]			
	MBC1	MOBC3	EBC191	BC10
BC	++	++	++	++
2C6MBC	+	−	−	−
EBC	++	++	++	++
MBC	++	++	+	++
MOBC	++	++	++	++
MN	−+	++	+	−
Adipodinitrile	+	−	+	+
Acrylamide	−	−	++	−
Lactamide	+	−	++	−
Lactonitrile	−	−	+	−
Succinodinitrile	+	−	++	++
Valeronitrile	++	−	−	+

[a] MBC1, MOBC3 and EBC191 *Pseudomonas fluorescens* MBC1, MOBC3 and EBC191; BC10 *Pseudomonas putida* BC10

Abbreviations: BC Benzylcyanide; 2C6MBC 2-chloro-6-methylbenzylcyanide; EBC α-ethylbenzylcyanide; MBC α-methylbenzylcyanide; MN mandelonitrile; MOBC α-methoxybenzylcyanide

The strains were grown with glucose (10 mM) and the respective nitrile (1 mM) initially used during enrichment. The cultures were transferred (1 : 10 v/v) to Erlenmeyer flasks with glucose (10 mM) and the nitrogen source tested (3 mM). The cultures were incubated at 30 °C on a rotary shaker. Growth was measured with a Klett-photometer

++ The cultures reached within one week an optical density ($OD_{546\,nm}$) higher than 0.96 or showed a significantly exponential growth curve

+ The cultures reached an optical density ($OD_{546\,nm}$) between 0.72–0.96

− The optical density ($OD_{546\,nm}$) was lower than 0.72

The controls without a nitrogen source added reached $OD_{546\,nm} < 0.36$

None of the strains grew with acetamide, acetonitrile, acrylnitrile, benzamide, butyronitrile, isobutyronitrile, malonodinitrile, methacrylamide, methacrylnitrile, propionamide or propionitrile as nitrogen source

always correlated with the disappearance of O-acetylmandelonitrile. O-Acetylmandelic acid and O-acetylmandelic acid amide were not further metabolized. When the composition of the enantiomers was assayed it became evident that always R(−)-O-acetylmandelic acid was formed in excess to S(+)-O-acetylmandelic acid. The amounts of products formed and the ratio of R(−)-O-acetylmandelic acid to S(+)-O-acetylmandelic acid are shown in Table 4.

Hydrolysis by cell-free extracts

Resting cells generated various amounts of O-acetylmandelic acid and O-acetylmandelic acid amide from O-acetylmandelonitrile. Therefore the preferential production of R(−)-O-acetylmandelic acid could be due to either enantioselective nitrilases, nitrile hydratases or amidases. Actually, the ability to hydrolyse O-acetylmandelonitrile was also detectable in cell-free extracts from *P. putida* BC10 or *P. fluorescens* strains MOBC3 and EBC191. In general the compounds formed by cell-free extracts and the relative amounts of them were similar to the products excreted by resting cells. Cell-free extracts from all strains converted O-acetylmandelonitrile to O-acetylmandelic acid, but did not attack O-acetylmandelic acid amide (or O-acetylmandelic acid).

Resting cells and cell-free extracts from *P. putida* BC10, *P. fluorescens* strain EBC191 and strain MBC1 formed mandelic acid from O-acetylmandelonitrile. In the case of *P. fluorescens* MOBC3 mandelic acid was formed only by crude extract but not by resting cells. Either O-acetylmandelonitrile or O-acetylmandelic acid could be subject to deacetylation. Cell-free extracts from all isolates were inactive with O-acetylmandelic acid as substrate. Thus it was concluded, that deacetylation could only occur at the level of O-acetylmandelonitrile as substrate.

Cell-free extracts from *P. fluorescens* MBC1 and MOBC3 were incubated with mandelonitrile (same reaction conditions as described with O-acetylmandelonitrile). Both cell-free extracts hydrolysed mandelonitrile completely to mandelic acid and benzoate. For *P. fluorescens* MBC1 a specific activity of 1.4 U/mg of protein was found. In a control experiment no spontaneous reaction of mandelonitrile was observed.

Table 4. Turnover of O-acetylmandelonitrile (AMN) by resting cells of different strains of the genus *Pseudomonas*

Pseudomonas strains[a]	AMN-turnover ($mM \times h^{-1}$)[b]	Products (%)[c]		Ratio of enantiomers (S : R)
BC10	1.71	AMA	(23)	1 : 10
		AMAA	(10)	n.d.
		MA	(23)	1 : 1
		BA	(50)	−
MBC1	1.14	AMA	(40)	1 : 3
		AMAA	(25)	n.d.
		MA	(20)	2 : 1
MOBC3	12.9	AMA	(80)	1 : 2
		AMAA	(10)	n.d.
EBC191	2.39	AMA	(40)	1 : 8
		AMAA	(21)	n.d.
		MA	(42)	5 : 1

Cells were grown with glucose (10 mM) and benzylcyanide (1 mM), afterwards harvested by centrifugation and resuspended to a final optical density $OD_{546\,nm}$ of 10 in Na/K-phosphate buffer (54 mM, pH 7.4). The flasks with the resting cells (10 ml culture in 100-ml Erlenmeyer flasks) were incubated on a rotary shaker at 30 °C. After addition of O-acetylmandelonitrile (3 mM) aliquots were taken at different time intervals, cells removed by centrifugation and the supernatants analyzed by reversed phase HPLC and using the Chiralcel OD-column

n.d. not determined

[a] BC10 *Pseudomonas putida* BC10; MBC1, MOBC3 and EBC191; *Pseudomonas fluorescens* MBC1, MOBC3 and EBC191

[b] maximum initial turn-over rate with resting cells ($OD_{546\,nm} = 10$)

[c] maximum concentrations reached, given in percent of AMN originally present

AMA O-acetylmandelic acid; AMAA O-acetylmandelic acid amide; BA benzoic acid; MA mandelic acid

Conversion of O-acetylmandelonitrile by cell-free extracts of Pseudomonas fluorescens MBC1

The experiments described above suggested an enantioselective hydrolysis of O-acetylmandelonitrile by a nitrilase resulting in the preferential synthesis of R(−)-O-acetylmandelic acid. In the experiments described above before chiral HPLC-analysis the samples were acidified and extracted into an organic phase (see 'Material and methods'). Under these conditions O-acetylmandelonitrile was not extracted into the organic layer. Therefore the enantioselective O-acetylmandelonitrile turnover could not be examined. To avoid this limitation O-acetylmandelonitrile was converted by a cell-free extract and extracted at neutral pH into the organic layer. The reaction-mixture contained 3 mg protein and 6 µmol O-acetylmandelonitrile in a total volume of 6 ml. After 3 min 45% of the R(−)-O-acetylmandelonitrile was converted, but only 18% of S(+)-O-acetylmandelonitrile. Thus it could be clearly demonstrated that indeed R(−)-O-acetylmandelonitrile was the preferred substrate.

Discussion

Bacteria capable of utilizing benzylnitriles such as benzylcyanide, α-ethylbenzylcyanide, α-methylbenzylcyanide or α-methoxybenzylcyanide (see Fig. 1) as sole nitrogen source seem to be present ubiquitously in nature. This may be explained by the fact that nitriles such as benzylcyanide and α-methylbenzylcyanide are natural products of plants, especially in the family *Cruciferae* (Bergstrom and Bergstrom 1989; Klepacka and Rutkowski 1982; Lockwood and Afsharypuor 1986; Hashimoto and Kameoka 1985).

Various reports exist on bacteria utilizing nitriles ether by nitrilases or by the combined action of nitrile hydratases plus amidases. Generally both groups of enzymes show a wide substrate range. Nitrilases usually are active with aromatic nitriles, but exhibit no or only marginal activity with aliphatic nitriles (Harper 1977a, b; Vaughan et al. 1988; Nagasawa et al. 1988b; Kobayashi et al. 1989). In contrast nitrile hydratases prefer aliphatic nitriles as substrates (Maestracci et al. 1984; Nagasawa et al. 1987; Watanabe et al. 1987; Nagasawa et al. 1988a; Endo and Watanabe, 1989; Tani et al. 1989).

The newly isolated strains converted O-acetylmandelonitrile to a mixture of O-acetylmandelic acid amide, O-acetylmandelic acid and mandelic acid. The potential biochemical reactions leading to these products are shown in Fig. 2. The present results strongly suggest that an enantioselective nitrilase is responsible for the synthesis of R(−)-O-acetylmandelic acid. This is supported by experiments with cell-free extracts which demonstrate that no amidase activity with O-acetylmandelic acid amide was present. The small amounts of amides formed during turnover of the corresponding nitriles could be explained by a side activity of the nitrilase. Amides as byproducts

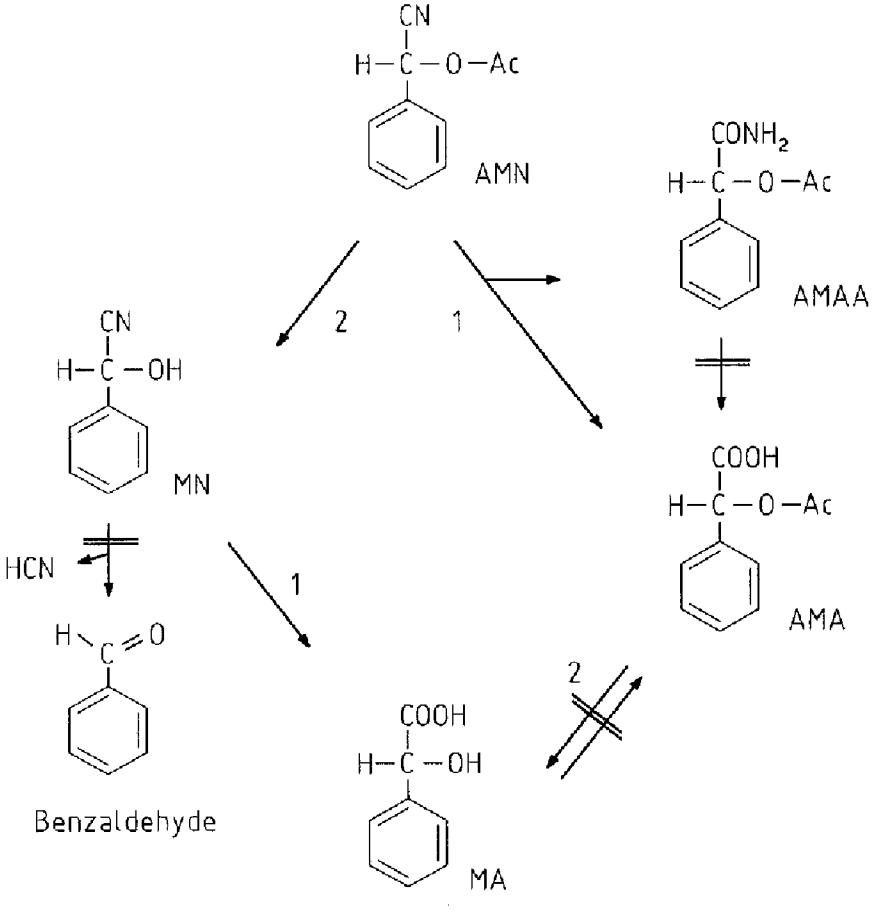

Fig. 2. Proposed pathway for the metabolism of O-acetylmandelonitrile by *Pseudomonas putida* BC10 and *Pseudomonas fluorescens* MBC1, EBC191 or MOBC3. AMN O-Acetylmandelonitrile; AMAA O-Acetylmandelic acid amide; AMA O-Acetylmandelic acid; MA Mandelic acid; MN Mandelonitrile. *1* Nitrilase; *2* esterase

have also been observed for ricine (N-methyl-3-cyano-4-methoxy-2-pyridone) nitrilase from a pseudomonad (Hook and Robinson 1964; Robinson and Hook 1964).

The formation of mandelic acid by resting cells and cell-free extracts suggested that also an esterase-activity in present in the bacteria. Since O-acetylmandelic acid or O-acetylmandelic acid amide were not deacetylated by cell-free extracts deacetylation must occur with O-acetylmandelonitrile as substrate.

The substrates converted by the nitrilases from the new isolates resemble those metabolized by the recently described arylacetonitrilase from *Alcaligenes faecalis* JM3, which was originally enriched with isovaleronitrile as nitrogen source, but also converted mandelonitrile and benzylcyanide (Mauger et al. 1990; Nagasawa et al. 1990).

Enzymatic reactions for the enantiospecific synthesis of mandelic acid or its derivatives have been described before. In the yeast *Rhodotorula graminis* and the bacterium *Lactobacillus curvatus* mandelate dehydrogenases form D(−)-mandelate from benzoylformate, whereas cells or *Streptococcus faecalis* IFO 12964 produce R(+)-mandelate (Baker and Fewson 1989; Hummel et al. 1988; Yamazaki and Maeda 1986a, b). The second, more general route to optically active aromatic α-hydroxycarboxylic acids is by using either (R)- or (S)-oxynitrilases. These enzymes from plant origin catalyze the stereospecific addition of HCN to an aldehyde resulting in either of the corresponding (R)- or (S)-α-hydroxycarboxylic acid (cyanhydrine) (Effenberger et al. 1987, 1990). There are several advantages using bacterial nitrilases for enantioselective synthesis. Dehydrogenases such as L- or D-mandelate dehydrogenases are restricted in their substrate range by the presence of an OH-group. The reaction catalyzed by oxynitrilases always results in the formation of α-hydroxysubstituted products. In contrast to these established systems nitrilases do not require any cofactors and offer the potential to use them for stereospecific synthesis of a wide range of chiral α-substituted carboxylic acids (Mayaux et al. 1990; Yamamoto et al. 1990; Yamamoto et al. 1991; Kakeya et al. 1991) from racemic substrates which are readily available by chemical synthesis.

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