Biodegradation **5**: 63–69, 1994.

Mineralization of 4-sulfophthalate by a *Pseudomonas* strain isolated from the River Elbe

Monika Richter[1] & Rolf-Michael Wittich[1,2,*]

[1]*Abteilung für Mikrobiologie, Institut für Allgemeine Botanik, Universität Hamburg, Ohnhorststrasse 18, D–2000 Hamburg 52, F.R. Germany;* [2] *Present address: Bereich Mikrobiologie, Gesellschaft für biotechnologische Forschung mbH, Mascheroder Weg 1, D–38124 Braunschweig, Germany (*Requests for offprints)*

Received 29 June 1993; accepted 19 October 1993

Key words: decarboxylation, desulfonation, 4,5-dihydroxyphthalate, *meta*-cleavage, protocatechuate pathway, *Pseudomonas* sp. RW31, 4-sulfophthalate mineralization

Abstract

The bacterium *Pseudomonas* sp. strain RW31 isolated from the river Elbe utilized the ammonium salt of 4-sulfophthalate (4SPA) as sole source of carbon, sulfur, nitrogen, and energy and grew also with phthalate (PA) and several other aromatic compounds as sole carbon and energy source. The xenobiotic sulfo group of 4SPA was eliminated as sulfite, which transiently accumulated in the culture supernatant up to about 10 μM and was slowly oxidized to the stoichiometrical amount of sulfate. Biodegradation routes of 4SPA as well as of PA converged into the protocatechuate pathway and from found activities for the decarboxylation of 4,5-dihydroxyphthalate we deduce this compound the first rearomaticized intermediate after initial dioxygenation. Protocatechuate then underwent *meta*-cleavage mediated by a protocatechuate 4,5-dioxygenase activity which was competitively inhibited by the structurally related compound 3,4,5-trihydroxybenzoate; protocatechuate accumulated in the medium up to an about 2 mM concentration. Indications for the presence of selective transport systems are presented.

Introduction

Sulfonated aromatics are found as structural elements of azo dyes and their respective building blocks, of detergents and of many other chemicals. Although the highly polar sulfo group renders this aromatic structure a very high recalcitrance against biological attack the mineralization of many sulfoaromatics has been described up to now (for references, cf. Hansen et al. 1992). Here, we report on the mineralization of 4-sulfophthalate (4SPA), which had been industrially used as a builder block in the production of tar dyes and which is currently used as a model compound in our studies.

Materials and methods

Isolation, identification and growth of the organism

4SPA degrading bacterial cultures were isolated by standard enrichment procedures from sediments and water samples of the Elbe river. For the identification of the strain standard laboratory methods were used. The strain RW31 has been deposited at the German Collection of Microorganisms and Cell Cultures (DSM), Braunschweig, F.R.G. (No. 7300). Cells were grown at 28 °C on a rotary shaker (150 rpm) in a mineral salts medium as described previously (Sander et al. 1991). Concentrations of aromatic substrates normally were 5 mM. For determinations of sulfite and sulfate the ammonium sulfate in the mineral salts solution was replaced by ammonium chloride.

Preparation of cell extracts, enzyme assays, and oxygen uptake measurements

The preparation of crude cell extracts, protein estimations and determinations of oxygen uptake rates were performed as previously described (Sander et al. 1991). Activities of the 3,4-dihydroxybenzoate 2,3-dioxygenase (Crawford 1975), 3,4-dihydroxybenzoate 3,4-dioxygenase (EC 1.13.1.3) (Stanier & Ingraham 1954), and 3,4-dihydroxybenzoate 4,5-dioxygenase (EC 1.13.1.8) (Ono et al. 1970) were performed by using these standard assays. For measurements of enzymic desulfonation in cell extracts two published methods were used (Endo et al. 1977; Thurnheer et al. 1986). Throughout this work the activity of the 4,5-dihydroxyphthalate decarboxylase was determined by following substrate consumption by HPLC for preventing those errors caused by superposition of UV spectra of substrate and products: using the standard assay conditions (Nakazawa & Hayashi 1978) aliquots were removed every 5 minutes, diluted with the same amount of icy-cold methanol, and chilled on ice for 1 h. Denatured protein was removed by centrifugation and the supernatant was subjected to HPLC analysis for quantitation of substrate concentrations.

Analytical methods and chemicals

Concentrations and in situ-scanned UV spectra of ethyl acetate-extracted aromatic compounds, as well as those in culture media or cell-free extracts were determined by high-pressure liquid chromatography (HPLC). The separations were performed on a reversed-phase column using acidified water (1 g of H_3PO_4/l) as the eluent. Thin layer chromatographic analysis of ethyl acetate-extracted compounds was performed as reported earlier (Sander et al. 1991). Sulfite and sulfate were estimated using previously described methods (Johnston et al. 1975; Bertolacini & Barney 1957). The technical mixture of sulfophthalates consisted of about 70% of 4-sulfophthalate and 30% of 3-sulfophthalate and was obtained from Aldrich-Chemie GmbH & Co. KG, Steinheim, F.R.G. 4-Sulfocatechol was synthesized from catechol (Quilico 1927). 3,4-Dimethoxybenzoate (Aldrich) served as the starting material for the synthesis of 4,5-dimethoxyphthalate (Edwards et al. 1925). Boron tribromide was used for cleavage of the methoxy groups (McOmie et al. 1968); 4,5-dihydroxyphthalate was further purified by preparative HPLC. Mineral salts and other chemicals were of the highest purity commercially available.

Results

Isolation, identification, and growth

Water and aerobic sediment samples from the Elbe river collected downstream of the city of Hamburg were used as inoculum for enrichment cultures. The slurry was supplemented with phosphate-buffered mineral salts and with the technical mixture of sulfophthalates (1 g/l). Increasing turbidity, disappearance of one of the two isomeric substrates, and the decline of the pH of the culture medium showed utilization of 4SPA as one of both isomerical target compounds. The concentration of 3-sulfophthalate in the enrichment cultures always remained unchanged. After about two months and several transfers into fresh medium a stable mixed culture consisting of three bacterial strains was obtained, which tentatively was named RW3. About three months later we isolated two organisms capable to grow with 4SPA and we have chosen one of them for our studies. This organism was termed RW31 and assigned to the genus *Pseudomonas* on the basis of the following characteristics: the Gram-negative, oxidase and catalase positive short rod (about 0.7 by 2.3 μm) grew only under strictly aerobic conditions. Motility was imparted by a pair of two polarly inserted flagella. Capsules or spores were not present. Colourless colonies were formed on solid selective medium. The guanine-plus-cytosine content of its DNA was 63.9 mol%.

The strain grew well in liquid culture at 20°C, 28°C, and 37°C but not at 4°C and 41°C and utilized the following substrates (5 mM each): 4SPA, PA, terephthalate, benzoate, 2-hydroxy-, 3-hydroxy- and 4-hydroxybenzoate, 2,5-dihydroxy-, 3,4-dihydroxy-, and 3,4,5-trihydroxybenzoate, 4-methylbenzoate, and catechol. The 3 isomeric sulfobenzoates, benzenesulfonate, aniline–2-sulfonate, 4-chloro- and 4-hydroxybenzenesulfonate, benzene–1,2- and benzene–1,3-disulfonate, toluene–4-sulfonat, 3-sulfophthalate, 5-sulfosalicylate, 4-sulfocatechol, 2,3-dihydroxy-, 2,4-dihydroxy-, and 2,3,4-trihydroxybenzoate, 4-methylphthalate, 2-methyl- and 3-methylbenzoate, isophthalate, 5-sulfoisophthalate, and 1,2,4-tricarboxybenzene representing the structural carboxyl analogue of 4-sulfophthalate, were not utilized for growth. The capability to utilize 4SPA was maintained after growth on acetate for 100 generations, indicating the stability of the organisms characteristic feature.

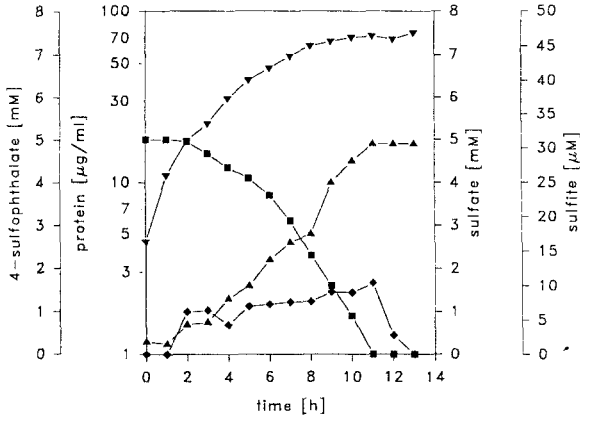

Fig. 1. Growth of *Pseudomonas* sp. RW31 on 4SPA as its sole source of carbon, sulfur, and energy. Growth (protein; ▼), substrate consumption (4SPA; ■), sulfite (◆), and sulfate (▲).

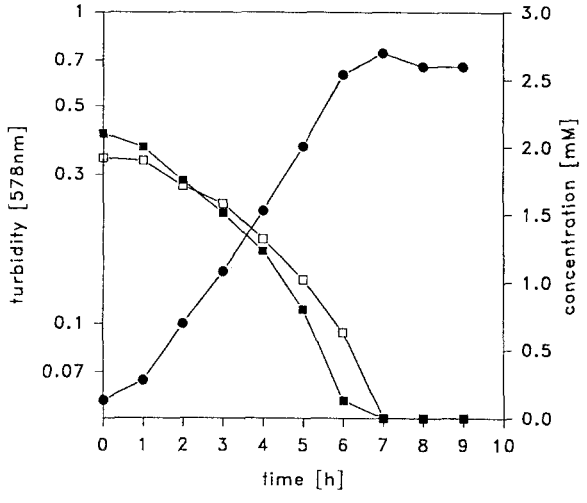

Fig. 2. Simultaneous growth of strain *Pseudomonas* sp. RW31 on 4SPA and phthalate. Growth (E_{578}; •), 4SPA (■), and PA (□).

Growth with 4-sulfophthalate and phthalate

Growth of *Pseudomonas* sp. RW31 with 4SPA as the sole source of carbon, sulfur, and energy is shown in Figure 1. In the absence of 4SPA growth was not observed and heat-inactivated 4SPA-grown cells (55 °C, 30 min) did not transform this compound. The doubling time was about 3.5 h. Sulfite ions were transiently released into the culture medium. Their concentration reached a maximum of 10 µM at the late logarithmic growth phase and these ions were stoichiometrically oxidized to sulfate. Similar results were obtained when the ammonium salt of 4SPA was utilized as a source of nitrogen instead of ammonium sulfate and the yield was about 18 g of protein per mol of utilized substrate in this experiment, providing evidence for the almost complete mineralization of the carbon skeleton of 4SPA. During growth of the organism, metabolites were not detectable by HPLC analysis of the culture medium. In the course of prolonged subcultivation, 4SPA concentrations of 20 mM were tolerated; however, the culture showed a significant lag phase. 3-Sulfophthalate was not attacked by our strain even after prolonged subcultivation over a period of about one year.

Strain RW31 also grew on PA (t_d, 1.5 h). In order to evaluate if both compounds compete for the same initial enzyme or transport system, strain RW31 was grown with an equimolar mixture of 4SPA and PA. Results shown in Figure 2 demonstrate that the consumption of 4SPA was concomitant with that of phthalate and the doubling time of this culture was 1.7 h.

The conversion of 4SPA and of PA by 4SPA-grown resting cells showed the immediate degradation of PA just starting several minutes after the complete utilization of 4SPA. A completely different observation was made when PA-grown cells were incubated in the presence of both PA and 4SPA. The turnover of the latter compound started about 2 h after the degradation of PA, indicating the induction of a probably new set of degradative enzymes. Results of these experiments are demonstrated in Figures 3 and 4. Interestingly, in the presence of equimolar concentrations of both 4SPA and protocatechuate, the latter compound was utilized after the complete consumption of 4SPA when resting cells had been pregrown with 4SPA (data not shown). In the course of the above experiments metabolites were not detectable in the culture supernatant by HPLC analysis.

Oxygen uptake rates and enzyme activities

Resting cells of our *Pseudomonas* sp. RW31 showed identical uptake rates for oxygen in the presence of PA and very similar rates for the oxidation of 4SPA, 4,5-dihydroxyphthalate, protocatechuate, and 3,4,5-trihydroxybenzoate irrespective of whether cells were grown on 4SPA or PA. With regard to the oxidation of 4SPA by PA-grown cells we found this observation not being correlated with the significantly delayed conversion of 4SPA by PA-grown cells. Acetate-grown

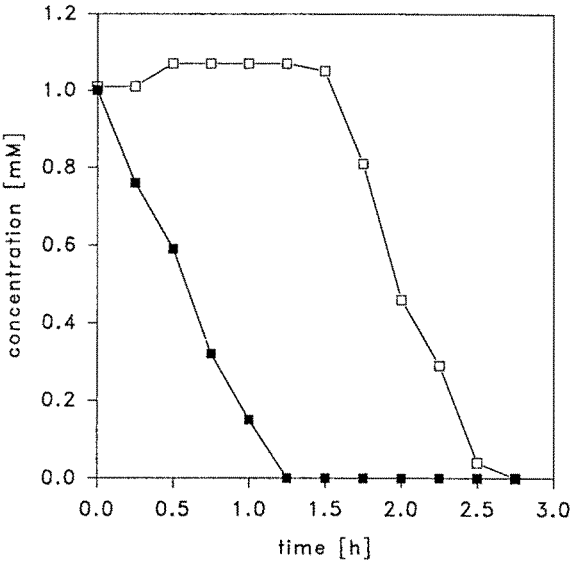

Fig. 3. Conversion of 4SPA (■) and PA (□) by resting cells of *Pseudomonas* sp. RW31 pregrown on 4SPA.

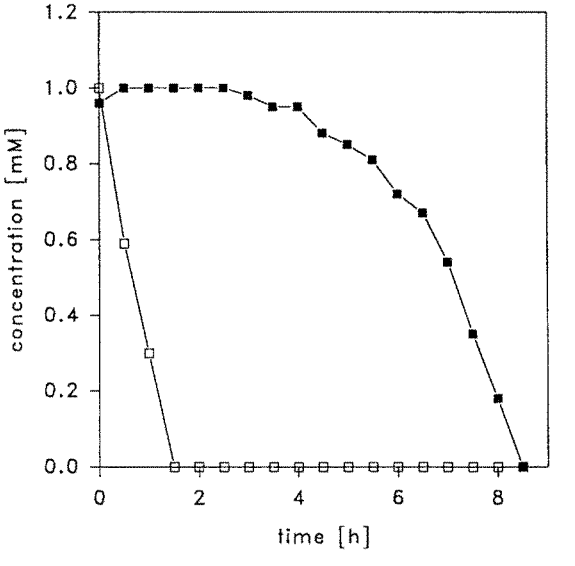

Fig. 4. Conversion of 4SPA (■) and PA (□) by resting cells of *Pseudomonas* sp. RW31 pregrown on PA.

resting cells exhibited a low level of about 10% of the activity of cells grown on the selective carbon source. None of the 14 further sulfoaromatics listet in Table 1 was oxidized by resting cells. Interestingly, extracts from cells grown on either the selective media or on acetate, but no resting cells slowly oxidized 4-

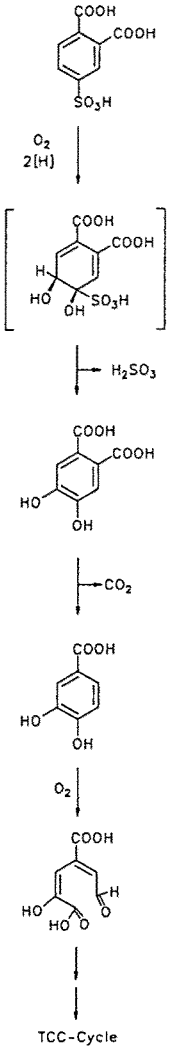

Fig. 5. Proposed pathway for the degradation of 4SPA by the strain *Pseudomonas* sp. RW31.

sulfocatechol. These extracts were also capable to oxidize 4,5-dihydroxyphthalate and protocatechuate.

Results from enzyme assays as demonstrated in Table 2 show significantly high activities for the turnover of 4,5-dihydroxyphthalate and for the activity of the protocatechuate 4,5-dioxygenase. No activities were detected in the protocatechuate 2,3-dioxygenase and protocatechuate 3,4-dioxygenase assay. A transient and very intense yellow color was observed during the conversion of protocatechuate, giving an indication for the *meta*-cleavage of the substrate. This colouring was also observed in the 4,5-dihydroxyphthalate decarboxylase assay. 3-Chlorocatechol, a potent inhibitor of those enzymes which catalyze the *meta*-

Table 1. Specific oxygen uptake rates of whole cells and cell-free extracts of the bacterium *Pseudomonas* sp. RW31.

Substrate	Specific oxygen demand (nmol O_2/min per mg protein) after growth with					
	4-Sulfophthalate		Phthalate		Acetate	
Phthalate	542	(< 5)	545	(< 5)	50	(< 5)
Isophthalate	< 5	(< 5)	< 5	(< 5)	< 5	(< 5)
Terephthalate	< 5	(< 5)	< 5	(< 5)	< 5	(< 5)
4-Carboxyphthalate	< 5	(nd)	< 5	(nd)	< 5	(nd)
3-Sulfophthalate	< 5	(< 5)	< 5	(< 5)	< 5	(nd)
4-Sulfophthalate	411	(< 5)	369	(< 5)	33	(< 5)
5-Sulfoisophthalate	< 5	(< 5)	< 5	(< 5)	< 5	(< 5)
4,5-Dihydroxyphthalate	134	(60)	161	(33)	18	(5)
4-Methylphthalate	14	(< 5)	34	(< 5)	< 5	(< 5)
Sulfobenzene	< 5	(nd)	< 5	(nd)	< 5	(nd)
1,2-Disulfobenzene	< 5	(nd)	< 5	(nd)	< 5	(nd)
1,3-Disulfobenzene	< 5	(nd)	< 5	(nd)	< 5	(nd)
2-Sulfoaniline	< 5	(nd)	< 5	(nd)	< 5	(nd)
4-Sulfophenol	< 5	(< 5)	< 5	(< 5)	< 5	(< 5)
4-Sulfocatechol	< 5	(19)	< 5	(38)	< 5	(13)
4-Sulfotoluene	< 5	(nd)	< 5	(nd)	< 5	(nd)
2-Sulfobenzoate	< 5	(nd)	< 5	(nd)	< 5	(nd)
3-Sulfobenzoate	< 5	(nd)	< 5	(nd)	< 5	(nd)
4-Sulfobenzoate	< 5	(nd)	< 5	(nd)	< 5	(nd)
5-Sulfo–2-hydroxybenzoate	< 5	(< 5)	< 5	(< 5)	< 5	(nd)
4-Sulfochlorobenzene	< 5	(< 5)	< 5	(< 5)	< 5	(< 5)
Benzoate	< 5	(nd)	< 5	(nd)	< 5	(nd)
2-Hydroxybenzoate	< 5	(< 5)	< 5	(< 5)	< 5	(< 5)
3-Hydroxybenzoate	14	(< 5)	7	(< 5)	< 5	(< 5)
4-Hydroxybenzoate	< 5	(< 5)	< 5	(< 5)	< 5	(< 5)
2,3-Dihydroxybenzoate	< 5	(< 5)	< 5	(< 5)	< 5	(< 5)
2,4-Dihydroxybenzoate	< 5	(< 5)	< 5	(< 5)	< 5	(< 5)
2,5-Dihydroxybenzoate	13	(< 5)	19	(< 5)	< 5	(< 5)
3,4-Dihydroxybenzoate	308	(116)	350	(58)	14	(8)
2,3,4-Trihydroxybenzoate	< 5	(< 5)	< 5	(< 5)	< 5	(< 5)
3,4,5-Trihydroxybenzoate	285	(50)	326	(32)	< 5	(< 5)
Catechol	8	(< 5)	38	(< 5)	13	(< 5)

Experiments were performed with washed cell suspensions grown on the respective substrates and cell-free extracts (data in parentheses). Uptake rates were corrected for endogenous oxygen consumption; concentrations of test compounds were between 0.1 mM and 1 mM and were always optimized for highest rates for individual substrates. Data represent means of at least three independently performed experiments; nd, not determined.

cleavage of catechol structures (Bartels et al. 1984; Klecka & Gibson 1981), however, only exhibited a very scarce activity.

Determinations of the K_m values of the protocatechuate 4,5-dioxygenase in cell extracts of 4SPA and PA-grown cells were 86 μM and 85 μM, respectively, and 3,4,5-trihydroxybenzoate found as an effective and competitive inhibitor of this ring cleaving dioxygenase revealed K_i data of 7.4 μM and 7.6 μM, in experiments performed with extracts of cells grown with 4SPA and PA, respectively.

Isolation and identification of metabolites

The identification of 3,4,5-trihydroxybenzoate as a potent inhibitor of the protocatechuate 4,5-

Table 2. Specific activities of catabolic enzymes from cell-free extracts of the strain *Pseudomonas* sp. RW31.

Enzyme	Spec. Activity (nmol/min per mg of protein) after growth with	
	4-Sulfophthalate	Phthalate
4,5-Dihydroxyphthalate decarboxylase	934	584
Protocatechuate 2,3-dioxygenase	< 1	< 1
Protocatechuate 3,4-dioxygenase	< 1	< 1
Protocatechuate 4,5-dioxygenase	1579	1019

Cells were grown in the presence of the appropriate carbon sources (5 mM) and worked up as described. The respective substrates were used in the assays. Data represent means of at least three independently performed experiments.

dioxygenase in an appropriate experiment allowed the isolation of protocatechuate from the culture supernatant of 4SPA-growing cells; the inhibitor concentration was 1 mM. The isolated compound co-chromatographed with authentical protocatechuate (HPLC); the in situ-scanned UV spectrum was also identical with that one of protocatechuate. Results were confirmed by TLC analysis of the ethyl acetate-extracted compound and the standard. Protocatechuate was also formed from 4,5-dihydroxyphthalate by extracts of both PA- and 4SPA-grown cells in the presence of 0.1 mM 3,4,5-trihydroxybenzoate. The oxidation and desulfonation of 4SPA, however, was not detectable in extracts of cells in our experiments. Metabolites were also not detectable in supernatants or solutions of the above experiments, nor they were in those experiments performed with 4SPA-grown resting cells in order to produce the carboxydihydrodiol from the structural analogue 1,2,4-tricarboxybenzene, on the analogy of similar experiments in which the carboxy analogues of sulfonated naphthalenes were co-oxidized to the respectively stable carboxydihydrodiols (Brilon et al. 1981; Wittich et al. 1988).

Discussion

The results from our experiments indicate that the transport of 4SPA into the bacterial cell obviously represents the first limiting step in the degradation of sulfoaromatics. The same observation already had been made earlier in the case of other mononuclear sulfonated aromatics (Thurnheer et al. 1990) and of other aromatic compounds carrying the highly polar dissociable carboxyl group (Groenewegen et al. 1990). We also presume that 4-sulfocatechol is not transported into the cell although it is oxidized by extracts according with the results from our oxygen uptake measurements. The oxidation should be due to the structural similarity with protocatechuate and we think that the narrow substrate specificity of the transport system for protocatechuate excludes the analoguous sulfocatechol. We, furthermore, have no indication neither for the probable *meta*-cleavage of 4-sulfocatechol nor for its intracellular desulfonation after ring cleavage (Feigel and Knackmuss 1988).

1,2,4-Tricarboxybenzene representing the structural analogue of 4SPA is not oxidized because it, perhaps, also is not transported into the cell due to the possibly very narrow substrate specificity of the transport system for 4SPA or, perhaps, of the limited substrate specificity of the sulfophthalate dioxygenase. Consequently, there is no proof for the dioxygenation of this tricarboxy compound and, therefore, we cannot show the specific region of the dioxygenolytic attack onto 4SPA: positions 3,4- und 4,5- are accessible which then could yield the respective intermediary and unstable sulfodihydrodiols in analogy to both stable dihydrodiols known from the degradation of phthalate (Ribbons & Evans 1960; Eaton & Ribbons 1982). Therefore, we cannot completely exclude 3,4-dihydroxyphthalate from the pathway we have suggested in Figure 5. A highly specific dioxygenase attacking and desulfonating 4-sulfobenzoate via a proposed, unstable sulfodihydrodiol has been characterized recently (Locher et al. 1991).

Found differences in specific enzyme activities for 4,5-dihydroxyphthalate decarboxylation and the *meta*-

cleavage of protocatechuate probably give evidences for the induction of separate sets of parallel pathways which means the expression of isofunctional enzymes for the degradation of 4SPA as well as for PA. Further studies are needed to understand the above observations as well as the discrepancy between the results from conversion experiments and those from oxygen uptake rates.

Acknowledgements

We thank Gaby Stehr for help in G + C content determination of chromosomal DNA. We thank Hans H. Locher and Hans R. Schläfli from the Institute of Microbiology, ETH Zürich, Switzerland, for samples of 3-sulfo- and 4-sulfobenzoic acid, respectively, and Victor Wray from GBF, Braunschweig, for running the ^{1}H NMR spectrum of our synthesized 4,5-dihydroxyphthalate.

References

Bartels I, Knackmuss H-J & Reineke W (1984) Suicide inactivation of catechol 2,3-dioxygenase from *Pseudomonas putida* mt–2 by 3-halocatechols. Appl. Environ. Microbiol. 47: 500–505

Bertolacini RJ & Barney JE (1957) Colorimetric determination of sulfate with barium chloranilate. Analyt. Chem. 29: 281–283

Brilon C, Beckmann W & Knackmuss H-J (1981) Catabolism of naphthalenesulfonic acids by *Pseudomonas* sp. A3 and *Pseudomonas* sp. C22. Appl. Environ. Microbiol. 42: 44–55

Crawford RL (1975) Novel pathway for degradation of protocatechuic acid in *Bacillus* species. J. Bacteriol. 121: 531–536

Eaton RW & Ribbons DW (1982) Metabolism of dibutyl phthalate and phthalate by *Micrococcus* sp. strain 12B. J. Bacteriol. 151: 465–467

Edwards GA, Perkin jun. WH & Stoyle FW (1925) New synthesis of the meconines. J. Chem. Soc. 127: 195–199

Endo K, Kondo H & Ishimoto M (1977) Degradation of benzenesulfonate to sulfite in bacterial extracts. J. Biochem. 82: 1397–1402

Feigel BJ & Knackmuss H-J (1988) Bacterial catabolism of sulfanilic acid via catechol–4-sulfonic acid. FEMS Microbiol. Lett. 55: 113–118

Groenewegen PEJ, Driessen AJM, Konings WN & De Bont JAM (1990) Energy-dependent uptake of 4-chlorobenzoate in the coryneform bacterium NTM–1. J. Bacteriol. 172: 419–423

Hansen C, Fortnagel P & Wittich R-M (1992) Initial reactions in the mineralization of 2-sulfobenzoate by *Pseudomonas* sp. RW611. FEMS Microbiol. Lett. 92: 35–40

Johnston JB, Murray K & Cain RB (1975) Microbial metabolism of aryl sulfonates, a re-assessment of colorimetric methods for the determination of sulphite and their use in measuring desulfonation of aryl and alkylbenzene sulphonates. Antonie van Leeuwenhoek 41: 493–511

Klečka GM & Gibson DT (1981) Inhibition of catechol 2,3-dioxygenase from *Pseudomonas putida* by 3-chlorocatechol. Appl. Environ. Microbiol. 41: 1159–1165

Locher HH, Leisinger T & Cook AM (1991) 4-Sulphobenzoate 3,4-dioxygenase. Purification and properties of a desulphonative two-component enzyme system from *Comamonas testosteroni* T–2. Biochem. J. 274: 833–842

McOmie JFW, Watts ML & West DE (1968) Demethylation of aryl methyl ethers by boron tribromide. Tetrahedron 24: 2289–2292

Nakazawa T & Hayashi E (1978) Phthalate and 4-hydroxyphthalate metabolism in *Pseudomonas testosteroni*: purification and properties of 4,5-dihydroxyphthalate decarboxylase. Appl. Environ. Microbiol. 36: 264–269

Ono K, Nozaki O & Hayaishi O (1970) Purification and some properties of protocatechuate 4,5-dioxygenase. Biochim. Biophys. Acta 220: 224–238

Quilico A (1927) Azione dell'acido amminosolfonico sui difenoli. Gaz. Chim. Ital. 57: 793–802

Ribbons DW & Evans WC (1960) Oxidative metabolism of phthalic acid by soil *Pseudomonads*. Biochem. J. 76: 310–318

Sander P, Wittich R-M, Fortnagel P, Wilkes H & Francke W (1991) Degradation of 1,2,4-trichloro- and 1,2,4,5-tetrachlorobenzene by *Pseudomonas* strains. Appl. Environ. Microbiol. 57: 1430–1440

Stanier RY & Ingraham JL (1954) Protocatechuic acid oxidase. J. Biol. Chem. 210: 799–808

Thurnheer T, Köhler T, Cook AM & Leisinger T (1986) Orthanilic acid and analogues as carbon sources for bacteria: growth physiology and enzymic desulphonation. J. Gen. Microbiol. 132: 1215–1220

Thurnheer T, Zürrer D, Höglinger O, Leisinger T & Cook AM (1990) Initial steps in the degradation of benzene sulfonic acid, 4-toluene sulfonic acid, and orthanilic acid in *Alcaligenes* sp. strain O–1. Biodegradation 1: 55–64

Wittich R-M, Rast HG & Knackmuss H-J (1988) Degradation of naphthalene–2,6- and naphthalene–1,6-disulfonic acid by a *Moraxella* sp. Appl. Environ. Microbiol. 54: 1842–1847