Letters in Applied Microbiology 1997, **24**, 474–478

The utilization of organosulphonates by soil and freshwater bacteria

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1299/96: received 17 September 1996 and accepted 6 November 1996

J.E. KING AND J.P. QUINN. 1997. Utilization of the biogenic aliphatic organosulphonates taurine, isethionate, sulphoacetaldehyde and sulphoacetate was investigated in 100 soil and freshwater bacteria isolated on modified complete mineral salts medium. More than 90% could use all the compounds as sole sulphur sources, and some 10% used taurine and isethionate as sole carbon and energy, or sole carbon, energy and sulphur sources. None could mineralize sulphoacetaldehyde or sulphoacetate; however, two isolates capable of growth on sulphoacetate as sole carbon, energy and sulphur source were obtained by enrichment culture. The results suggest that in the majority of environmental bacteria the pathways of organosulphonate biodegradation may be independently controlled by the supply of carbon and sulphur to the cell, and that a number of routes may exist for cleavage of the organosulphonate C–S bond.

INTRODUCTION

Organosulphonates contain a direct carbon–sulphur (C–S) bond in which the sulphur atom is at an oxidation state of +4. Their environmental fate is important in the understanding of the global sulphur cycle, in which they participate in an intermediary position between the sulphate ion (+6) and sulphide (−2) (Seitz *et al*. 1993)

A variety of natural organosulphonates is present in diverse organisms, e.g. taurine (2-aminoethanesulphonate), isethionate (2-hydroxyethanesulphonate), coenzyme M (2-mercaptoethanesulphonate), and the plant sulpholipid, 1,2-diacyl-3-(6-sulpho-a-D-quinopyrosyl)-L-glycerol (reviewed by Seitz and Leadbetter 1995). Sulphur in synthetic C–S compounds is commonly found in the form of sulphonate groups bound to an aromatic nucleus, as in the linear alkylbenzene sulphonates (LAS) or the sulphonated dyestuffs. LAS dominate in the cleaning industry and are among the most common components of domestic and industrial wastestreams.

The C–S bond of the organosulphonates has great chemical stability (Wagner and Reid 1931) and this is reflected in the increased recalcitrance which the addition of a sulphonate group gives to a previously biodegradable molecule (Cain 1981). Vairavamurthy *et al*. (1994) have reported the accumulation of organosulphonates in near-surface marine sediments to a level of 20–40% of the total organic sulphur, while sulphonate sulphur has been shown to exceed 40% of total S in the O1 horizon of 17 out of 18 forest soils examined (Autry and Fitzgerald 1990).

The ability to mineralize organosulphonates as a carbon and energy source seems to be confined to a relatively few bacterial species; however, the utilization of the sulphonate–sulphur appears to be a more widespread trait (Seitz and Leadbetter 1995). The extent of information on the biodegradation of the different classes of organosulphonates is very uneven. Details of the microbial assimilation of the aliphatic organosulphonates, such as taurine, are few and incomplete (Uria-Nickelsen *et al*. 1993), whereas the biodegradation of the synthetic aromatic organosulphonates has been studied in a variety of bacteria (Lee and Clark 1993).

With this in mind, the aim of the study was to obtain bacterial isolates capable of utilizing the aliphatic organosulphonates taurine, isethionate, sulphoacetate and sulphoacetaldehyde (an intermediate of taurine and isethionate breakdown) as sole sulphur, sole carbon and energy, or sole carbon, energy and sulphur sources, and to clarify the abundance in some soil and freshwater environments of bacteria with these biodegradative capabilities. Two approaches were

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adopted, a non-selective screening process and an enrichment technique.

MATERIALS AND METHODS

Chemicals

All chemicals, with the exception of hydroxymethylsulphonate and sulphoacetaldehyde, were obtained from Sigma/Aldrich and were of the highest purity available. Hydroxymethylsulphonate was a gift from Dr L. E. Hallas (Monsanto Co., St Louis, MO, USA). Sulphoacetaldehyde was synthesized, as the bisulphite addition complex, by QUCHEM, The Queens's University of Belfast, UK, using the method of Kondo *et al*. (1971). For use a stock solution was prepared in distilled water, and 2 equivalents of HCl were added. The sample was then degassed with N_2 until all the SO_2 was expelled (about 30 min). Sodium bicarbonate (2 equivalents) was then added (White 1988). A fresh sample was prepared daily. Stock solutions of all organosulphonates were tested for the presence of contaminating sulphate by the method of Sorbo (1987) and any sulphate present was precipitated by the addition of equimolar amounts of $BaCl_2$.

Growth of organisms

Complete mineral salts medium (CMSM) (pH 7·2) contained, per litre of distilled water, KCl, 0·2 g; $MgSO_4.7H_2O$, 0·2 g; $CaCl_2.2H_2O$, 1·0 mg; NH_4Cl, 1·0 g; sodium gluconate, 2·5 g; sodium pyruvate, 2·5 g; ferric ammonium citrate, 1·0 mg; phosphate buffer 5 mmol l^{-1}; yeast extract, 0·05 g; and 1 ml each of $\times 1000$ solutions of trace elements (Krieg 1981) and vitamins (Difco). When used for isolation of environmental bacteria it was modified by the replacement of sulphate by chloride salts and the omission of yeast extract and trace elements, and was prepared using tap water with a sulphate content of approximately 10 mg l^{-1}. For screening of isolates for organosulphonate utilization, tap water was replaced by distilled deionized water. Solid medium was prepared by the addition of either Bactoagar (Difco) or Oxoid High Purity Agar as per experimental requirements, each at a concentration of 1·2% w/v.

Flasks (250 ml) containing 30 ml of filter-sterilized medium were incubated on a rotary shaker (100 rev min^{-1}) at 27°C. Cell growth was measured by the increase in $O.D._{650}$ using a PU 8200 UV/Vis. spectrophotometer (Pye-Unicam Ltd, Cambridge, UK). Sulphate levels in culture supernates were measured by the method of Sorbo (1987).

The non-selective isolation and screening of soil and water bacteria

Samples (1 g) of soil, water or lakeside mud, taken from seven sites, both urban and rural, within Northern Ireland, were added individually to 9 ml of 0·85% (w/v) sterile saline and serial dilutions prepared. An aliquot (50μl) of each was plated on modified CMSM and incubated at 27°C for 5 days, after which 100 morphologically distinct bacterial isolates were selected and transferred to fresh plates on which they were routinely maintained.

Bacterial isolates were subsequently replica-streaked on solidified sulphur-free medium containing either: (a) 0·1 mmol l^{-1} taurine, isethionate, sulphoacetate or sulphoacetaldehyde as sole sulphur source, (b) 10 mmol l^{-1} of one of the sulphonates as sole carbon and energy source plus 0·1 mmol l^{-1} Na_2SO_4 as a sulphur source, (c) 10 mmol l^{-1} of one of the sulphonates as sole carbon, energy and sulphur source, or (d) no sulphonate supplementation. The plates were incubated for up to 5 days, and isolates subcultured to fresh plates on two further occasions. Subsequent growth of any isolates unable to utilize an organosulphonate supplied as sole carbon, energy and sulphur source was negligible and invariably indistinguishable from that on carbon- and sulphur-free medium. Growth of isolates on solidified sulphur-free medium was also barely visible (cf. Seitz *et al*. 1993) and permitted those isolates able to utilize an organosulphonate supplied as sole sulphur source to be unambiguously distinguished by eye in all but a few instances. In these latter cases results were confirmed in liquid culture; growth was scored as positive if the optical density of a culture in which an organosulphonate was supplied as sole sulphur source was more than five times that on sulphur-free medium. The ability of selected bacterial isolates to utilize a further eight aliphatic and aromatic organosulphonates as sole sources of carbon and energy, or as sole sulphur sources, was determined in liquid culture in which the organosulphonates were supplied at concentrations of 10 mmol l^{-1} and 0·1 mmol l^{-1}, respectively.

Enrichment for organosulphonate-mineralizing bacteria

Enrichment culture was carried out in modified CMSM in which 10 mmol l^{-1} taurine, isethionate, sulphoacetate or sulphoacetaldehyde replaced gluconate and pyruvate as sole carbon and energy source, using a 0·5% (v/v) mixed inoculum from an activated sludge plant at Dunmurry Sewage Works (Belfast) and a laundromat waste disposal lagoon (Summit Lake, Wisconsin, USA).

After 10 serial transfers 50-μl aliquots of an appropriate serial dilution of each liquid culture were plated on solidified enrichment medium and incubated for 7 d at 27°C. In all instances only a few morphologically distinct colony types were present; pure cultures of those strains judged to be predominant were obtained from individual colonies.

RESULTS

Screening of environmental bacteria for utilization of aliphatic organosulphonates

Of the 100 isolates, 47 came from soil sites, 39 from water samples and 14 from freshwater sediment. Table 1 lists the percentage of bacterial isolates capable of utilizing each aliphatic organosulphonate under each nutritional limitation. Most isolates could utilize all four organosulphonates as sole sulphur source; however, only a few were capable of using any of the four as sole carbon and energy source and then only taurine and isethionate were mineralized. No isolate could utilize sulphoacetate or sulphoacetaldehyde as sole carbon and energy source. Those bacteria capable of utilizing either taurine or isethionate as sole carbon and energy source could (with one exception) also use the same compounds as sole carbon, energy and sulphur sources. All those isolates that could utilize isethionate as sole carbon and energy source could also use taurine in the same capacity. The fact that faint background growth, comparable to that on 'no substrate' control plates, was observed in the case of all isolates in the presence of organosulphonates that they did not utilize suggests that none was toxic to cells.

Isolation of organosulphonate-mineralizing bacterial strains by enrichment

After 10 serial transfers, enrichment cultures yielded two Gram-negative rods that each utilized taurine as sole carbon and energy source, two isolates (one Gram-positive) that similarly utilized sulphoacetate, and a Gram-negative rod that utilized isethionate. All organisms grew with essentially stoichiometric sulphate release, accompanied by a sharp decline in culture pH. No strains capable of utilizing sulphoacetaldehyde as sole carbon and energy source were isolated.

Organosulphonate substrate-range of bacterial isolates

The utilization, by four of the bacterial isolates obtained through enrichment (above), of the organosulphonates taurine, isethionate, sulphoacetate and sulphoacetaldehyde, and of an additional eight aliphatic and aromatic sulphonates, is shown in Table 2. The substrate range of isolate NS1, a Gram-negative rod isolated from soil on complete CMSM medium, is also shown.

None of the bacteria tested could mineralize sulphoacetaldehyde, and isolate NS1 was unable to use any organosulphonate as sole carbon and energy source. Those bacteria obtained through enrichment on either taurine, isethionate or sulphoacetate could mineralize each of the other two compounds, however, suggesting that these might share a common degradative intermediate. (An exception was the Gram-positive strain SFCD2, which was isolated on sulphoacetate but unable to mineralize taurine.)

No strain utilized any of the eight additional organosulphonates tested as a sole source of carbon and energy, but each isolate used at least three of the compounds as sole sulphur source. Strains TCDB and SFCD1 could desulphonate all eight compounds under conditions of sulphur limitation; by contrast isolate NS1 utilized only primary aliphatic organosulphonates as a sulphur source.

DISCUSSION

The results of screening 100 environmental isolates indicate that the ability to desulphonate the aliphatic organosulphonates, for use as sole sulphur source, is a much more widespread trait than is their utilization as sole carbon and energy source (Table 1). The ability to cleave the organosulphonate C–S bond under conditions of sulphur limitation may well be controlled as part of a sulphate starvation-induced stimulon (Kertesz *et al.* 1994; Kertesz 1996) and would thus not lead to significant organosulphonate mineralization due to the excess sulphate that would result. However, some 10% of bacterial isolates could utilize the sulphonates taurine and isethionate as sole sources of carbon, energy and sulphur (Table 1), while two strains that similarly mineralized sulphoacetate were obtained by enrichment (Table 2). This finding supports the proposed existence of C–S bond-cleaving isoenzymes whose expression is independently regulated as part of the carbon and sulphur cycles (Kertesz 1996). It

Substrate provided as sole source of	Organosulphonate substrate			
	Taurine	Isethionate	Sulphoacetate	Sulphoacetaldehyde
Sulphur	94	96	92	97
Carbon	11	9	0	0
Carbon and sulphur	10	9	0	0

Table 1 The number of 100 randomly selected environmental bacterial isolates capable of utilizing aliphatic organosulphonates as sole sources of sulphur, carbon and energy, or carbon, energy and sulphur

Table 2 Utilization of organosulphonates as sole carbon and energy source (C), or sole sulphur source (S), by environmental bacterial isolates

	Isolates											
	TCDB*		ICD†		SFCD1‡		SFCD2‡		NS1§		*Comamonas acidovovans* I 91‖	
Organosulphonate	C	S	C	S	C	S	C	S	C	S	C	S
Taurine	+	+	+	+	+	+	−	−	−	+	+	+
Isethionate	+	+	+	+	+	+	+	+	−	+	+	+
Sulphoacetate	+	+	+	+	+	+	+	+	−	+	ND	ND
Sulphoacetaldehyde	−	+	−	+	−	+	−	+	−	+	ND	ND
Methanesulphonate	−	+	−	+	−	+	−	+	−	+	−	+
Ethanesulphonate	−	+	−	+	−	+	−	−	−	+	ND	ND
Hydroxymethylsulphonate	−	+	−	+	−	+	−	+	−	+	ND	ND
Sulphosuccinate	−	+	−	+	−	+	−	+	−	−	ND	ND
Benzenesulphonate	−	+	−	−	−	+	−	+	−	−	ND	ND
Naphthalene-1-sulphonate	−	+	−	+	−	+	−	+	−	−	ND	ND
Sulphanilate	−	+	−	−	−	+	−	+	−	−	ND	ND
Metanilate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	−	−
p-Toluenesulphonate	−	+	−	−	−	+	−	+	−	+	−	+

* Isolated on basis of ability to mineralize taurine.
† Isolated on basis of ability to mineralize isethionate.
‡ Isolated on basis of ability to mineralize sulphoacetate.
§ Isolated on complete mineral salts medium.
‖ Data obtained from Seitz *et al*. (1993).
Growth is scored as '+' or '−' where '−' is equivalent to the level of growth in the appropriate sulphonate-free control.
ND, Not determined.

is consistent with a previous report that six environmental bacterial strains, including *Comamonas acidovorans* I91, which were originally isolated for their ability to utilize the organosulphonates taurine and isethionate as sole sources of carbon, energy and sulphur, subsequently lost the ability to use these compounds as sole carbon (though not as sole sulphur) sources, after storage on rich medium (Seitz *et al*. 1993).

Of the eight additional sulphonates tested (Table 2), none was utilized as sole carbon and energy source. All isolates could use a selection of the compounds as sole sulphur sources, however ; it is likely that they were able to enter the cell since no evidence of extracellular bacterial C–S bond cleavage activity has been reported. Aliphatic molecules were more widely metabolized ; for example the random soil isolate NS1 could utilize only primary aliphatic organosulphonates. Taken together with the comparatively narrow substrate range of, for example, strain ICD it seems unlikely that a single broad-spectrum, sulphur-regulated desulphonating activity is responsible.

Our results shed new light on the abundance of soil and freshwater bacteria capable of the complete mineralization, or the desulphonation, of organosulphonates. They further demonstrate the likelihood of the existence of at least two routes for cleavage of the organosulphonate C–S bond, depending on whether the carbon- or sulphur-containing moiety of the molecule is required by the cell. A more complete knowledge of these activities and of their regulation would greatly improve our understanding of the mineralization of organosulphonates within the global sulphur cycle.

ACKNOWLEDGEMENTS

The authors thank Rabih Jaouhari of QUCHEM, Queens's University of Belfast, for the synthesis of sulphoacetaldehyde. The work was supported by a Postgraduate Award from the Department of Education for Northern Ireland.

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