Microbiology (1995), 141, 2897–2904 Printed in Great Britain

Arylsulphatase from *Alteromonas carrageenovora*

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Arylsulphatase activity was identified in cultures of the marine bacterium *Alteromonas carrageenovora*, using methylumbelliferyl sulphate as substrate. In contrast with most other microbial arylsulphatases, arylsulphatase production in *A. carrageenovora* was not repressed by sulphate. The structural gene of arylsulphatase (*atsA*) was cloned and sequenced. An ORF of 984 bp was found, specifying a primary translation product of 328 amino acids with a molecular mass of 35797 Da. Arylsulphatase was partially purified from cell extracts of both *A. carrageenovora* and recombinant *Escherichia coli*. Both the recombinant and native enzymes exhibited a pI of 5·5, a Michaelis constant for methylumbelliferyl sulphate of 68 μM, and a molecular mass of approximately 35000 Da in SDS-PAGE analysis. Secondary structure comparisons using hydrophobic cluster analysis suggest functional analogies between the arylsulphatase of *A. carrageenovora*, that of *Mycobacterium leprae* and a 33·5 kDa protein from *Porphyromonas gingivalis*. It is speculated that these proteins are all glycosulphohydrolases, involved with desulphatation of sulphated polysaccharides.

Keywords: *Alteromonas carrageenovora*, arylsulphatase, glycosulphohydrolase, hydrophobic cluster analysis

INTRODUCTION

Arylsulphatase (aryl-sulphate sulphohydrolase, EC 3.1.6.1), an enzyme involved with the metabolism of organic sulphur and sulphate, catalyses the hydrolysis of phenolic ester sulphates such as methylumbelliferyl sulphate (MUF-S) or *p*-nitrophenyl-sulphate, as follows: $H_2O + \text{Aryl-O-}SO_3^- \leftrightarrow \text{Aryl-OH} + SO_4^{2-} + H^+$.

In aerobic soils, which are poor in inorganic sulphate, arylsulphatases are thought to provide bacteria with sulphate, by catalysing the hydrolysis of such arylsulphate esters as 2-hydroxy-5-nitrophenylsulphate and phenyl-sulphate from humic and fulvic acids (Fitzgerald, 1976). In both soil bacteria, such as *Pseudomonas* strain C12B (George & Fitzgerald, 1981), and enteric bacteria, such as *Klebsiella aerogenes* (Rammler *et al.*, 1964; Adachi *et al.*, 1975; Murooka *et al.*, 1978; Oka *et al.*, 1980) and *Salmonella typhimurium* (Henderson & Milazzo, 1979), as well as in the green alga *Chlamydomonas reinhardtii* (De Hostos *et al.*, 1988), expression of arylsulphatase is strongly inhibited by the presence of sulphate or organic sulphur. In the marine environment, which is abundant in sulphate ions, an arylsulphatase activity not inhibited by sulphate was reported in the bacterium *Alcaligenes metalcaligenes* (Fitzgerald, 1976). In addition an arylsulphatase, active on phloroglucinol sulphate esters and partially inhibited by sulphate, was purified to homogeneity from the brown alga *Cystoseira tamariscifolia* (Knöss & Glombitza, 1993).

The enzyme from *Pseudomonas* strain C12B was purified to homogeneity (George & Fitzgerald, 1981) and the arylsulphatase gene from *K. aerogenes* was cloned and sequenced (Murooka *et al.*, 1990). Several eukaryotic arylsulphatase genes have also been described. They include arylsulphatases from the sea urchins *Hemicentrotus pulcherrimus* (Sasaki *et al.*, 1987, 1988) and *Strongylocentrotus purpuratus* (Yang *et al.*, 1989), and from *C. reinhardtii* (De Hostos *et al.*, 1989). In addition nucleotide sequences of other sulphatases with arylsulphatase activity are known, e.g. the human lysosomal arylsulphatases A (cerebroside-sulphatase, EC 3.1.6.8) (Stein *et al.*, 1989a), and B (*N*-acetylgalactosamine-4-sulphatase, EC 3.1.6.12) (Peters *et al.*, 1990) as well as human arylsulphatase C (steryl-

Abbreviations: ASW, artificial sea water; HCA, hydrophobic cluster analysis; IEF, isoelectric focalization; MUF, 4-methylumbelliferone; MUF-S, 4-methylumbelliferyl sulphate.

The EMBL and Swiss-Prot accession numbers for the sequence reported in this paper are X65709 and P28607 (ARS_ALTCA), respectively.

sulphate sulphohydrolase, EC 3.1.6.2), a membrane-bound, microsomal enzyme (Stein *et al.*, 1989b; Yen *et al.*, 1987).

We report here on the presence of a functionally active arylsulphatase in *Alteromonas carrageenovora*, a marine aerobic Gram-negative bacterium (Akagawa-Matsushita *et al.*, 1992) known to hydrolyse carrageenans, the sulphated galactans of red algae (Johnston & McCandless, 1973; Weigl *et al.*, 1966; Yaphe & Baxter, 1955; McLean & Williamson, 1979a), and to desulphate oligo kappa-carrageenans (Weigl & Yaphe, 1966; McLean & Williamson, 1979b, 1981). We describe the cloning, nucleotide sequence and expression of the arylsulphatase of *A. carrageenovora* and discuss its possible functional relationships with two proteins identified in pathogenic bacteria involved with degradation of human tissues, *Mycobacterium leprae* and *Porphyromonas gingivalis*.

METHODS

Bacterial strains and culture conditions. *A. carrageenovora* (ATCC 43555) was obtained from the American Type Culture Collection and was grown in Zobell Medium (Zobell, 1941) at 25 °C, or in artificial sea water (ASW), consisting of 25 g NaCl l^{-1}, 0·5 g $MgCl_2$ l^{-1}, 0·1 g $CaCl_2$ l^{-1}, 1 g KCl l^{-1}, 1 mg $FeCl_2$ l^{-1} and 12 mM phosphate buffer (pH 8·5). When appropriate ASW was supplemented with 5 g galactose l^{-1}, 1 g NH_4Cl l^{-1} or 1 g $NaNO_3$ l^{-1}, 1 mM cysteine or Na_2SO_4. *E. coli* strain DH5α[*recA1 endA1 gyrA96 thi1 hsdR17* (r_K^- m_K^+), *supE44 relA1 lacZ* ΔM15] was grown on Luria–Bertani medium (LB) at 37 °C (Maniatis *et al.*, 1982). When appropriate, ampicillin (LBA) or tetracycline (LBT) was added to solid or liquid medium at 50 μg ml^{-1} or 15 μg ml^{-1}, respectively.

Cloning and sequencing of the arylsulphatase gene. Preparation of the genomic library from *A. carrageenovora* was described previously (Barbeyron *et al.*, 1994). The recombinant clones were inoculated for 18 h at 37 °C in microtitration plates in LBA medium, supplemented with MUF-S (50 μg ml^{-1}). Wells containing positive arylsulphatase clones exhibited fluorescence under UV light (366 nm), owing to the release of 4-methyl-umbelliferone (MUF) into the culture medium.

Plasmid DNA was isolated by the alkaline lysis method (Birnboim & Doly, 1979) and mapped with restriction endonucleases. Restricted fragments from two of the positive recombinant plasmids (pSA3 and pSA4) were recovered onto Na 45 Schleicher & Schuell DEAE nylon paper and subcloned into phagemid pBluescriptII KS(−) (Stratagene). Sequencing was carried out using the dideoxy-sequencing method (Sanger *et al.*, 1977). Sequence similarities were investigated throughout the NCBI data bank and further analysed using the hydrophobic cluster analysis program (HCA) (Dorian).

Arylsulphatase assay. The MUF-S stock solution was made in formamide at the concentration of 25 mg ml^{-1} and stored at 4 °C in the dark. Aliquots of homogenous cell suspensions or cell extracts (5–20 μl) were mixed with 1 ml reaction buffer (20 mM Tris/HCl, pH 8·5, 500 mM NaCl, 10 mM $CaCl_2$), supplemented with 50 μg (170 nmol) MUF-S and MUF production was measured at 360 nm. Enzyme activities are expressed in nmol MUF released per min.

Production of arylsulphatase by cultures of *A. carrageenovora* and *E. coli* recombinant clones was estimated as follows: the OD_{580} of stationary-phase cultures was measured and the culture medium (10 ml) was centrifuged at 2000 ***g*** for 10 min; supernatants were concentrated 10-fold. The bacterial pellet was washed and resuspended with 1 ml reaction buffer. Aliquots (100 μl) of cell suspensions and of concentrated supernatants were mixed to 0·9 ml with reaction buffer supplemented with 45 μg MUF-S, and assayed for production of MUF. To allow for comparison between different culture conditions, enzyme activies were referred to the OD_{580} of the initial culture.

Partial purification and analysis of arylsulphatases. Pellets from 3 l cultures of *A. carrageenovora* grown overnight in Zobell medium were lysed with a French press (Aminco) at 13 MPa and extracted in 50 mM Tris/HCl, pH 7·2, 10 mM $MgCl_2$, 10 mM NaCl and 3 mM $CaCl_2$. Debris were removed by centrifugation at 10000 ***g***. Proteins from 100 ml *E. coli* cultures grown overnight in LBA medium supplemented with 50 mM Tris/HCl, pH 7·2, 10 mM $MgCl_2$, 5 mM KCl, 3 mM $CaCl_2$, with or without 25 μg MUF-S ml^{-1} were extracted as described above.

Crude cell extracts from either *A. carrageenovora* or recombinant *E. coli* were mixed with Heparin-Sepharose CL 6B (Pharmacia) suspended in 25 mM Tris/HCl pH 8·5, 5 mM $CaCl_2$, 1 mM benzamidin and 1 mM PMSF. The affinity phase was packed into a column (1 × 5 cm) and proteins were eluted by Tris/HCl buffer (25 mM, pH 7·5), containing 1 M NaCl,1 mM benzamidin and 1 mM PMSF. Sulphatase activity in the eluent was monitored with 25 μg MUF-S ml^{-1}. The active fractions were pooled, de-salted and applied to a MonoQ HR 5/5 column (Pharmacia), pre-equilibrated with 20 mM Tris/HCl, pH 8·5. The column was eluted with a gradient of NaCl (0–500 mM) in 20 mM Tris/HCl, pH 8·5. Upon chromatography through the MonoQ column, arylsulphatase activity from *A. carrageenovora* fell into two distinct fractions, at 150 mM (fraction I) and 200 mM NaCl (fraction II), in proportions of about 1 : 2. Further fractionation and analysis of native arylsulphatase were carried out with fraction II. In *E. coli* recombinant clone pSXS3, only one arylsulphatase fraction was present, eluted at 200 mM NaCl.

The sulphatase fractions were concentrated 20-fold and analysed by SDS-PAGE (Laemmli, 1970), using a PhastSystem (Pharmacia). SDS-polyacrylamide gels were stained with silver nitrate. Non-denaturing PAGE was performed using stacking gels and resolving gels with 6% (w/v) and 30% (w/v) acrylamide, respectively. After migration, the native gels were soaked with 100 mM Tris/HCl, pH 8·0, first for 10 min then for 3 min in the same solution containing 25 μg MUF-S ml^{-1} and MUF fluorescence was observed under UV light. The pI of the sulphatase was measured with an isoelectric focalization (IEF) gel using a PhastSystem (Pharmacia) developing a 3–9 pH range. Two IEF gels were run in parallel, one was stained with silver nitrate and the other was incubated with MUF-S.

Effect of salts and pH on arylsulphatase activity and K_m determinations. The effect of sodium chloride concentration on arylsulphatase activity was estimated in Tris/HCl buffer (20 mM, pH 8·0) over the range 0–1·5 M NaCl. Effects of potassium, magnesium and calcium chloride were examined in the same buffer over the range 0–50 mM. The influence of pH was measured over the range pH 5·5–11·5 in 500 mM NaCl and 10 mM $CaCl_2$ buffered with 50 mM sodium acetate, pH 5·5, 50 mM MES buffer (pH 5·5–6·5) 50 mM MOPS (pH 6·5–7·5), 50 mM Tris/HCl (pH 7·2–10·0) and 50 mM Tris-base (pH 11·5). Michaelis constants (K_m) were determined using Hanes' plots with substrate concentrations ranging from 0·01–400 μM in 20 mM Tris buffer, pH 8·0, containing 500 mM NaCl and 10 mM $CaCl_2$.

RESULTS

In 24-h-old cultures of *A. carrageenovora* MUF was readily detected upon addition of MUF-S, indicating the presence in the bacterium of an enzyme capable of hydrolysing

Table 1. Influence of culture conditions on production of arylsulphatase by *A. carrageenovora*

Cultures were initiated with the same inocula, cells were recovered at the stationary phase and supernatants and cells were assayed for sulphatase activity. Measurements were performed in triplicate and experiments were repeated once, yielding a SD below 1%.

Medium*	Bacterial OD_{580}†	Total activity (nmol min^{-1})††	Activity (nmol min^{-1} OD^{-1})
Zobell medium: supernatant		0·024	0.01
Zobell medium	2·90	2·210	0·76
Zobell medium + MUF-S	2·89	2·000	0·69
ASW + Gal + NH_4Cl	0·71	0·262	0·36
ASW + Gal + $NaNO_3$	0·13	0·410	3·01
ASW + Gal + NH_4Cl + Cys	1·81	0·575	0·31
ASW + Gal + NH_4Cl + Na_2SO_4	0·56	0·415	0·74
ASW + Gal + $NaNO_3$ + Na_2SO_4	0·24	0·275	1·14

* ASW, artificial sea water; Gal, 1 g galactose l^{-1}; 1 g NH_4Cl l^{-1}; 1 g $NaNO_3$ l^{-1}; Na_2SO_4, 1 mM; Cys, 1 mM cysteine.

† Stationary-phase cultures.

†† In the bacterial pellet, unless stated otherwise.

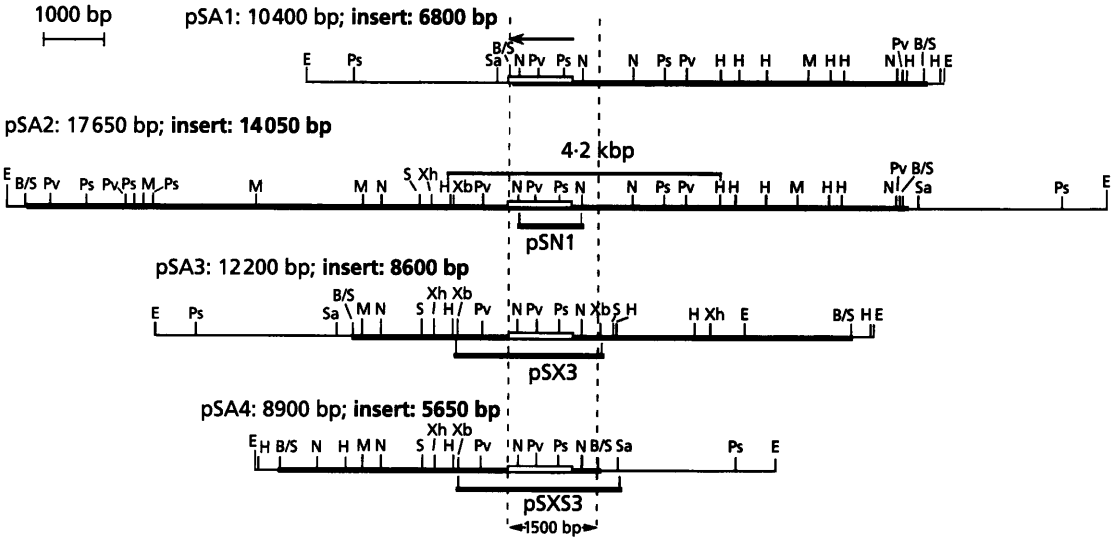

Fig. 1. Physical maps of *A. carrageenovora* genomic clones with arylsulphatase activity. Thin lines indicate the pAT153 regions, bold segments refer to *A. carrageenovora* inserts and open boxes show the arylsulphatase gene. The direction of transcription is indicated by the arrow. Dashed lines delimit the region common to all of the positive clones. The *Nde*I, *Xba*I and *Xba*I–*Sal*I fragments, that yielded pSN1, pSX3 and pSXS3 pBluescript subclones, respectively, are shown by bold segments below the restriction maps. The *Hin*dIII fragment used for Southern hybridization is delineated above the pSA2 map. Restriction sites: E, *Eco*RI; B/S, *Bam*HI–*Sau*3A1 junction; Ps, *Pst*I; Pv, *Pvu*II; M, *Mlu*I; N, *Nde*I; H, *Hin*dIII; Xb, *Xba*I; Xh, *Xho*I; S, *Sma*I; Sa, *Sal*I.

phenolic ester-sulphates. The activity was found in intact bacterial pellets as well as in cell lysates, whereas no significant arylsulphatase activity was detected in culture supernatants. Compared to cultures in ASW with no sulphur source, production of arylsulphatase was not inhibited when bacteria were grown in the presence of cysteine or Na_2SO_4. Activity per OD_{580} unit was highest with $NaNO_3$ as sole nitrogen source. Culturing in the presence of MUF-S did not enhance the production of arylsulphatase (Table 1).

Cloning of the arylsulphatase gene

Approximately 5000 genomic clones were obtained, with a background of clones without inserts of 17%. On the basis of the appearance of a luminous spot under UV

Table 2. **Production of arylsulphatase by the various recombinant *E. coli* clones grown in LBA medium**

Arylsulphatase activity was assayed in cells from stationary-phase cultures. Measurements were performed in triplicate and experiments were repeated once, yielding a SD below 1 %.

Plasmid	Bacterial OD_{580}	Total activity (nmol min^{-1})	Activity (nmol min^{-1} OD^{-1})
pSA1	5·93	0·20	0·03
pSA2	5·68	6·47	1·14
pSA3	5·16	3·43	0·66
pSA4	5·37	8·19	1·52
pSN1	7·33	0·00	0·00
pSXS3	3·91	8·54	2·18
pSX3	5·04	9·70	1·90

light, four clones were identified as being sulphatase positive. They are referred to as pSA1–4.

Plasmid DNA was isolated from the four arylsulphatase clones and mapped with restriction endonucleases (Fig. 1). Inserts ranged from 5·6 to 14·0 kbp. The four plasmids shared a common *Nde*I fragment of 1·0 kbp. Plasmids pSA3 and pSA4 were used for subcloning experiments. Only the subclones harbouring the *Xba*I fragment (3·0 kbp) from pSA3 or the *Sal*I–*Xba*I fragment (3·0 kbp) from pSA4, which both contained the *Nde*I fragment, were capable of degrading MUF-S. The resulting plasmids are referred to as pSX3 and pSXS3, respectively.

Arylsulphatase clones were investigated for expression of sulphatase activity upon culture in liquid LBA medium (Table 2). Compared to *A. carrageenovora*, relative expression of arylsulphatase activity in clones pSA1–4 and subclones pSXS3 and pSX3 was 0·04, 1·5, 1·0, 2·0, 3·0 and 2·6, respectively. Plasmid pSN1, which consisted of the *Nde*I fragment only (Fig. 1), did not break MUF-S down, indicating that the arylsulphatase gene is not entirely encompassed by this fragment.

Biochemistry of native and recombinant arylsulphatases

Arylsulphatase was partially purified from cell extracts of *A. carrageenovora* and of *E. coli* pSXS3 clone, by affinity chromatography on Heparin-Sepharose CL 6B followed by ion-exchange chromatography. The native (fraction II) and recombinant enzymes were identical in their kinetics and behaviour towards protons and salts. For both the *A. carrageenovora* and the recombinant *E. coli* partially purified arylsulphatase fractions, the K_m for MUF-S was reproducibily measured at 68 µM and sulphatase activity displayed a broad optimum at pH 8·5 and reached a plateau for NaCl concentrations above 500 mM. Addition of KCl, $MgCl_2$ or $CaCl_2$ had no effect on arylsulphatase activity. Non-denaturing PAGE of the partially purified arylsulphatase fractions followed by incubation in the fluorogenic substrate showed for both *A. carrageenovora* and *E. coli* pSXS3 clone the presence of one fast-moving

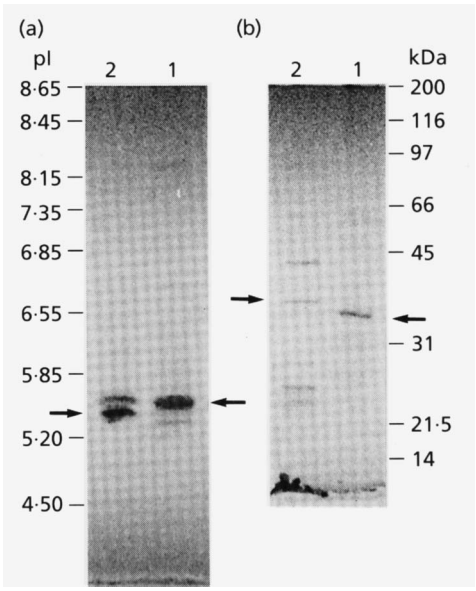

Fig. 2. **Biochemical analysis of the arylsulphatases partially purified from cell extracts of *A. carrageenovora* (fraction II) (lane 1) and from *E. coli* pSXS3 subclone (lane 2). (a) IEF gel stained with silver nitrate. The major bands (arrows) appear at approximately pH 5·5. Incubation of a similar gel with MUF-S revealed a broad fluorescent band corresponding to the major protein bands. (b) SDS-PAGE gel stained with silver nitrate. The major bands (arrows) appear at approximately 34·0 and 36·0 kDa.**

protein band, which became fluorescent upon incubation in the presence of MUF-S (not shown). IEF electrophoresis followed by staining with silver nitrate and detection with MUF-S also showed a similar pI for native and recombinant arylsulphatase, at approximately 5·5 (Fig. 2a). In *A. carrageenovora*, SDS-PAGE of the partially purified arylsulphatase fraction showed the presence of a major protein band, at approximately 34·0 kDa, and of a minor band, at 36·0 kDa (Fig. 2b). In *E. coli* pSXS3 clone, an inversed pattern was obtained with the major band at 36·0 kDa and the minor one at 34·0 kDa.

Nucleotide sequence analysis of the arylsulphatase gene

Plasmids pSN1 and pSXS3 were sequenced in both strands, to determine the nucleotide sequence of the arylsulphatase gene. Translation of all six reading frames of the nucleotide sequence of the 2·28 kbp *Sal*I–*Nde*I region contained within the 3·0 kbp *Sal*I–*Xba*I fragment revealed only one ORF of 984 bp, which covers the region starting 142 bp after the first *Nde*I site up to 117 bp beyond the second *Nde*I site (Fig. 3). The G+C content of the sulphatase sequence, 39·7 mol %, falls within the range of G+C-content values characteristic of the genus *Alteromonas* (Baumann *et al.*, 1972; De Vos *et al.*, 1989), showing that the gene composition is not biased with respect to the whole genome. The sequence upstream of the start codon contains three regions that share some

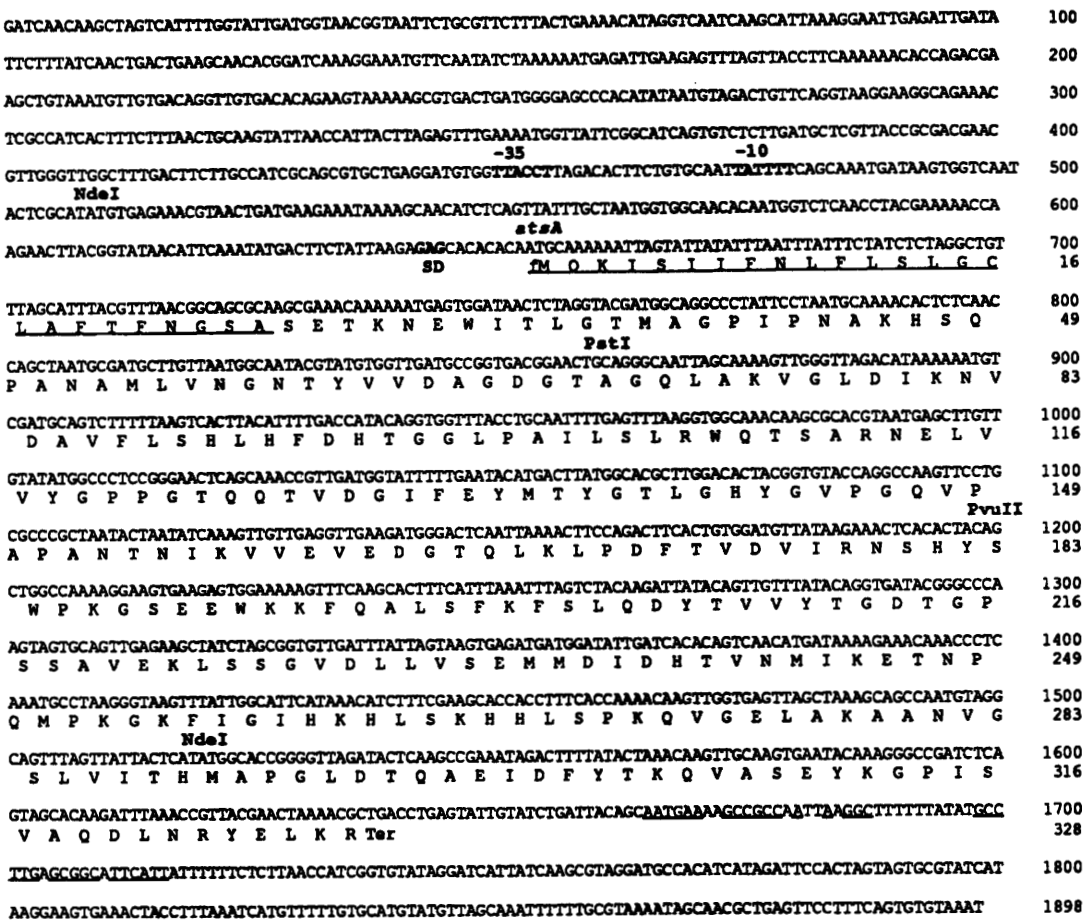

Fig. 3. Nucleotide and deduced amino acid sequences of the arylsulphatase gene from *A. carrageenovora* (*atsA*). The coding region starts at nucleotide 652, which is 142 bp downstream from the first *Nde*I site, and ends at nucleotide 1635, 117 bp downstream of the second *Nde*I site. Putative '−35' and '−10' sequences for the promoter and the potential Shine–Dalgarno (SD) sequence are indicated in bold. The six T residues starting at nucleotide 1718 are likely to be the transcription termination site. The regions with possible diad symmetry are underlined. A putative 25 amino acid signal peptide is underlined. Restriction sites are shown above the nucleotide sequence.

homology with known *E. coli* consensus sequences for promoters and RBSs. A putative Shine–Dalgarno sequence, GAG, is located 7 bp upstream from the ATG initiation codon. Upstream (160 bp) from this Shine–Dalgarno sequence, the TATttT and TTacCt sequences could correspond to the '−10' and '−35' of a putative promoter of the gene of *A. carrageenovora* (Harley & Reynolds, 1987). An area of stem–loop formation, 25 bp downstream of the TGA stop codon, may act as a site for transcription termination (Rosenberg & Court, 1979). The free energy associated with this stem–loop stucture is estimated to be −24·8 kcal mol^{-1} (−103·8 kJ mol^{-1}) (Tinoco *et al.*, 1973), a physiologically consistent value. Moreover, this hairpin structure is followed by six T residues, a sequence representative for a rho-independent termination site (Rosenberg & Court, 1979).

The predicted translation product of the sulphatase gene is a protein of 328 amino acid residues, with a theoretical molecular mass of 35797 Da. As indicated by hydropathy analysis (not shown), the NH_2-terminal end of the protein stands out as a domain of 25 amino acids with a high hydrophobicity. This domain is likely to represent the signal peptide (von Heijne, 1985), with peptidase processing of the pre-protein at the peptide bond between residues 25 (alanine) and 26 (serine).

The arylsulphatase from *A. carrageenovora* displays no significant sequence similarity with arylsulphatases from *Enterobacteriacae* (Murooka *et al.*, 1990), from the green alga *C. reinhardtii* (De Hostos *et al.*, 1989) or from sea urchins (Sasaki *et al.*, 1988; Yang *et al.*, 1989). It shares, however, some amino acid identity (25 and 20%, respectively) with the so-called arylsulphatase from *M. leprae* (GenBank U00014) as well as with a 33·5 kDa gene product of unknown function (referred to as 33·5 K.pep), from the periodontopathogenic bacterium *P. gingivalis* (G. Rigg, personal communication). As illustrated in Fig. 4, HCA did delineate several identical hydrophobic domains along the secondary structures of these proteins, notably from amino acid residues 80–100, 220–230 and 275–295 (numbering of the *A. carrageenovora* sequence).

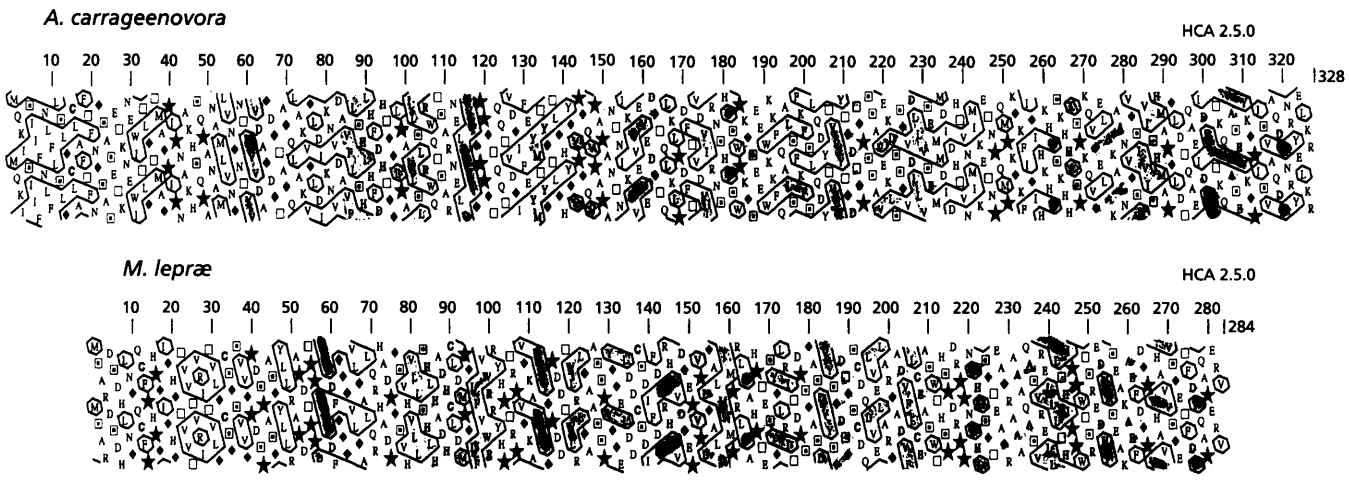

Fig. 4. **HCA plots of arylsulphatases from *A. carrageenovora* and *M. leprae*. In these plots, the clusters formed by contiguous hydrophobic residues are drawn. Shaded areas highlight the conserved hydrophobic clusters, delineating analogous domains, notably from residues 80–100, 220–230, and 275–295 (numbering of *A. carrageenovora*). The topologically conserved hydrophilic amino acids are shaded.**

DISCUSSION

As shown by the breaking down of MUF-S by cell pellets and extracts, *A. carrageenovora* synthesizes an arylsulphatase. In arylsulphatase assays with unbroken bacterial pellets, MUF was readily detected in the reaction medium. However, the enzyme was not present in significant amounts in culture supernatants. These observations suggest a periplasmic-space or an external-membrane location of the protein, as described for *Pseudomonas* strain C12B (Fitzgerald & George, 1977). This is consistent with hydropathy analysis of the protein deduced from the nucleotide sequence, which shows the presence of a signal peptide.

We have identified the structural gene of the arylsulphatase from *A. carageenovora* by functional cloning into *E. coli*. To our knowledge this is the first report of the gene sequence of an arylsulphatase from a marine bacterium. The recombinant enzyme had kinetic characteristics identical to the native protein. Rather than showing the existence of a second arylsulphatase gene, the differences observed in the physical maps of pSA2 and pSA3 (Fig. 1) are probably due to ligation of two different *Sau*3A1 inserts in the latter plasmid. This hypothesis was confirmed by Southern blot analysis (data not shown), in which the arylsulphatase probe hybridized with one 4·2 kbp *Hin*dIII fragment only, consistent with the pSA2 map. Compared to *A. carrageenovora*, overproduction of arylsulphatase by pSA2, pSA4, pSXS3 and pSX3 is likely to be due to a high copy-number of recombinant vectors. By contrast, the pSA1 clone exhibited a 20-fold shift-down of the activity, suggesting that sequences downstream of the arylsulphatase gene positively regulate its expression. Similarly, pSA3 did not overproduce arylsulphatase, showing that the putatively artefactual insert upstream of the gene reduced the production of the protein in this clone. Translation of the ORF results in a protein of 35·8 kDa. Partially purified arylsulphatase fractions from recombinant *E. coli* contained a major protein band, with an apparent molecular mass of 36·0 kDa, consistent with the above value, as well as a minor band with a slightly smaller molecular mass, about 34·0 kDa. In arylsulphatase from *A. carrageenovora*, however, a major band was detected by SDS-PAGE at 34·0 kDa whereas a minor band was detected at 36·0 kDa. Similarly, double bands with arylsulphatase activity were detected in IEF gels. These results as well as the presence of a signal peptide of 25 amino acids suggest that the primary translation product is processed down to a mature protein of 304 amino acids, with a theoretical molecular mass of 33100 Da. We assume that this processing was not as significant in the recombinant *E. coli* clone.

Similarly to the marine bacterium *Alc. metalcaligenes* (Fitzgerald, 1976), production of arylsulphatase in *A. carageenovora* is not repressed by sulphate ions or cysteine, a finding consistent with the presence of large amounts of sulphate in sea water. Since arylsulphatase from *A. carrageenovora* is probably not essential for providing the bacterium with sulphur, one may therefore ask what is the physiological function of this constitutive enzyme in *A. carageenovora*. A likely possiblity, consistent with the ecology of this bacterium, is involvement with desulphatation of seaweed compounds for use as carbon sources. However, no sulphohydrolase activity was observed upon incubation of brown algal phloroglucinol monosulphate and diphtorethol disulphate in the presence of *A. carrageenovora* arylsulphatase (W. Knöss & K.-W. Glombitza, personal communication). The arylsulphatase

from *A. carrageenovora* was also incapable either of removing sulphate from neocarrabiose-4-*O*-sulphate or from neocarratetraose-4-*O*-sulphate (data not shown), indicating that the enzyme is not functionally related to the 55 kDa glycosulphatase previously characterized in this bacterium (McLean & Williamson, 1979b). As *A. carrageenovora* was reported to also degrade sulphated fucans from brown algae (Yaphe & Morgan, 1959), we are currently assessing the possibility that sulphated oligofucans are the physiological substrate of arylsulphatase.

The protein sequence of arylsulphatase from *A. carrageenovora* displays no significant similarity to the microbial arylsulphatases repressed by sulphate (De Hostos *et al.*, 1989; Murooka *et al.*, 1990) nor to the animal proteins reported with arylsulphatase activity (Peters *et al.*, 1990; Sasaki *et al.*, 1988; Stein *et al.*, 1989a, b; Yang *et al.*, 1989). Interestingly, however, HCA plots indicate that the arylsulphatase of *A. carrageenovora* and two protein sequences, from the pathogenic bacteria *P. gingivalis* and *M. leprae*, respectively, share domains with identical 3-D foldings. Since the two latter bacteria both cause diseases that affect the extracellular matrix, it is tempting to speculate that these proteins are glycosulphohydrolases, involved with the breakdown of sulphated glycosaminoglycans.

ACKNOWLEDGEMENTS

We are grateful to Dr W. Knöss and Professor K.-W. Glombitza (Bonn) for assessing arylsulphatase for activity on algal sulphated polyphenols and to Dr G. Rigg (Leicester) for sharing unpublished sequence data. We thank G. Gugglielmi and C. Astier for helpful suggestions on the manuscript.

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Received 23 January 1995; revised 18 May 1995; accepted 30 June 1995.