Cultural Conditions of Arylsulfatase Activity in *Escherichia coli**

K. DHEVENDARAN**, D. CHANDRAMOHAN† and R. NATARAJAN
Centre for Advanced Studies in Marine Biology, Annamalai University, Porto Novo - 608 502

Arylsulfatase activity and growth were estimated in *Escherichia coli,* isolated from marine sediment. Maximum activity was observed at pH 6.6 whereas the maximum growth was at pH 5.6. 2×10^{-3} m M is the optimum substrate concentration for the highest level of enzyme activity/synthesis as well as for its growth. In general higher substrate concentration tended to inhibit enzyme activity and also the growth of the bacterium. Maximum growth and highest enzyme activity occurred at 29°C and above this temperature decreased both of them. Besides these, glucose, sodium sulfate, sodium chloride, sodium dihydrogen phosphate, sodium acetate and ammonium chloride at higher concentrations were inhibiting the enzyme activity and growth. Above 0.2% of glucose, 3% of sodium chloride, 10×10^{-3} M concentrations of sodium sulfate, sodium dihydrogen phosphate, sodium acetate and ammonium chloride inhibited the activity and growth also. These observations indicate that, to generalise a compound as inhibitor or activator it is difficult since this depends not only on its concentration but also on the source of the enzyme when more than one type is encountered in nature.

Arylsulfatase (Arylsulfate sulfohydrolase, E.C. 3.1.6.1) catalyses the hydrolysis of sulfuric acid esters of aromatic compound. There is an increasing awareness, now, of the importance of the part played by this enzyme. Arylsulfatase of various types are present in most animals, including mammals, birds, amphibia, marine mulluscs and polychaetes (Dodgson & Spencer, 1956, Dhevendaran *et al.* 1980, Dhevendaran, 1984) and also in barnacles (Shimony & Nigrelli, 1972). No extensive study on the distribution of arylsulfatase among bacteria appears to have been made. But the presence of this enzyme in some bacteria has also been reported particularly in *Salmonella* spp. and *Mycobacterium* spp. by Whitehead *et al.* (1952), in some unidentified marine bacteria by Chandramohan, *et al.* (1974) and in *Vibrio* spp., *Pseudomonas* spp. and *Bacillus* spp. Dhevendaran *et al.* (unpublished) isolated it from the primary film and foulers. Besides these, detailed investigations have been made on the nature and activity of arylsulfatase in *Aerobacter aerogenes* (Harada 1957, Fowler & Rammler, 1964, Adachi *et al.* 1973) in *Proteus vulgaria* (Dodgson, 1959) in *Pseudomonas aeruginosa* (Harada, 1964) and in *Proteus rettgeri* (Milazzo & Fitzgerald, 1967). Interestingly, from the marine environment, only a few strains of microorgansims especially a yeast, *Trichosporon cutaneum* and two bacteria, such as *Alcaligenes metalcaligenes* and *Mycobacterium piscium* exhibited this enzyme activity. Evidently little attention has so far been paid to study the arylsulfatase activity in other bacterial genera from the marine sources. The aim of the present study is concerned with the arylsulfatase activity in *E. coli* isolated from the marine sediment and the influence of a variety of culture conditions on arylsulfatase biosynthesis and growth.

Materials and Methods

Dodgson & Spencer (1957) studied arylsulfatase activity in bacteria by the detection of phenolphthalein liberated from tripotassium phenolphthalein disulfatese (PDS) which was incorporated in the medium. Same principle was followed by Dhevendaran (1978) and the culture was identified by the method of Shewan, *et al.* (1960).

*Formed part of the Ph. D. Thesis of the first author approved by Annamalai University

**Present address: Department of Aquatic Biology and Fisheries, University of Kerala, Trivandrum

†Present address: National Institute of Oceanography, Goa - 403 004

For the determination of arylsulfatase activity of growing *E. coli* culture, a modified method of Whitehead, *et al.* (1952) as employed by Dodgson *et al.* (1954) was followed. Centrifuge tubes graduated at 10 ml were dried at 105°C and 1.0 ml of 0.02 M PDS was added to each tube. Control tubes containing 1.0 ml water were prepared. The tubes were plugged with cottonwool and autoclaved for 20 min at 15 lb. No denaturation of the substrate occurred under these conditions. Sterile nutrient broth (Peptone: 1.0 g, Beef-extract 0.5 g, Nacl 0.5 g, 50% seawater, 100 ml, pH 7.1) was added to each tube to give a total volume of 10 ml and after incubation overnight at 37.5°C any tube showing growth was rejected. The remaining tubes were inoculated with 2 drops of an activity growing cultures (10^8cells/ml) and incubated at room temperature (28 ± 2°C). Tubes were tested after 48 h as follows:

The volume of liquid in each tube was adjusted to 16 ml with sterile water to compensate for loss due to evaporation, and the bacteria were separated by centrifugation at 15,000 g, 9 ml of clear supernatant was mixed with 1.0 ml of 1 N NaOH and the red phenolphthalein colour was measured spectrophotometrically at 560 nm, using the control tubes as blanks. The concentrations of phenolphathalein in unknown samples were calculated from a standard curve prepared with authentic sample of phenolphthalein. The growth of the bacteria culture was measured following the method of Lowry *et al.* (1951).

Results and Discussion

Previous reports show that arylsulfatase activity is common in members of the family Enterobacteriaceae (Whitehead, *et al.* 1952) Milazzo & Fitzgerald, 1966). In some of the strains of *E. coli* tested, Harada *et al.* (1954) could observe weak phenolsulfatase activity with p. nitrophenyl sulfate (NPS) but not with PDS. Compared to that the present culture differed markedly in arylsulfatase activity and exhibited a very specific substrate specificity towards PDS but not with NPS. The distribution of *E. coli* in marine sediments has been observed by earlier workers (Selvakumar *et al.* 1977). Arylsulfatase activity in growing cultures was studied using the nutrient broth with PDS as the substrate. For studies on pH, cells were grown in media buffered with either 0.1 M acetate or 0.1 M phosphate salts at different pH values for 48 h. It is evident from the results (Fig. 1) that maximum growth occurred at pH 5.6 and the highest level of arylsulfatase was found at pH 6.6, thereby indicating that arylsulfatase synthesis was not influenced by growth but by the pH of the medium. A similar observation has been reported (Milazzo & Fitzgerald, 1967) in *Proteus rettgeri,* where most rapid rate of growth occurred at pH 6.6 and the highest level of arylsulfatase was found at pH 7.0 similar to Dhevendaran *et al.* (1980) and Dhevendaran (1984). When the pH of the

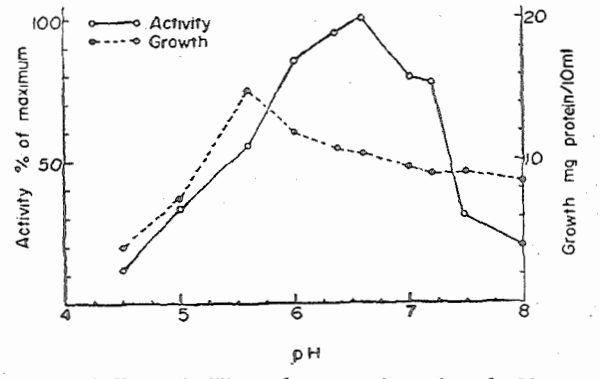

Fig. 1. Effect of pH on the growth and arylsulfatase activity of *E. coli*.
PDS–100% activity = 24.60 ug phenolphthalein liberated/mg protein/h

medium was raised to 8, only 20% of maximum activity was observed. This indicates that, under natural conditions, the production of arylsulfatase must be at a very low level, since the pH of the seawater and sediment ranged from 7.9 to 8.3 normally (Dhevendaran, 1978).

The effect of substrate concentration on the growth and arylsulfatase production was also studied and the results (Fig. 2) indicate that maximum production of enzyme was at a substrate concentration of 2×10^{-3}M. As the concentration of the substrate increased enzyme production decreased. Even an increase of the substrate concentration from 2×10^{-3}M to 3×10^{-3}M resulted in a loss of 32% of maximum activity. When the concentration was increased to 20×10^{-3} M almost complete loss of enzyme synthesis could be noticed. It is thus evident that growth was not affected to any appreciable extent at a concentration of 10×10^{-3}M but further increase resulted in a drastic

reduction in the growth rate. Such inhibition of enzyme activity and growth by excess substrate concentration is not unique

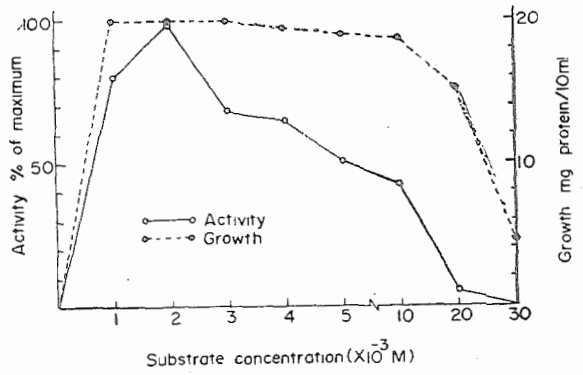

Fig. 2. Effect of substrate concentration on the growth and arylsulfatase activity of *E. coli* PDS–100% activity = 24.80 ug phenolphthalein liberated/mg protein/h

(Dixon & Webb, 1958, Webb, 1963). To know effect of temperature on growth and arylsulfatase synthesis, the culture was maintained at six different temperatures. The results (Fig. 3) show that although growth and enzyme synthesis could be noticed in the temperature range of 20°C and 40°C, there were differences in rate of growth and

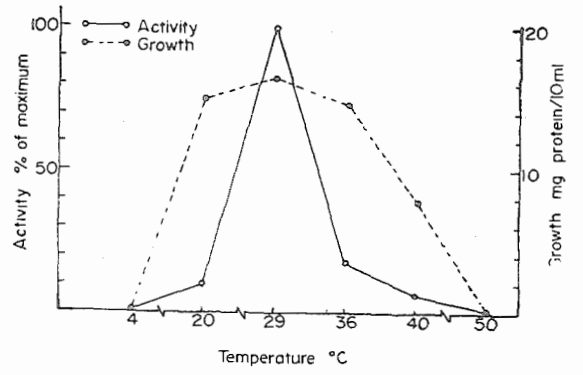

Fig. 3. Effect of temperature on the growth and arylsulfatase activity in *E. coli* PDS–100% activity = 24.37 ug phenolphalein liberated/mg protein/h

in enzyme activity. Maximum growth and highest enzyme activity towards PDS occurred at 29°C. Temperature above 29°C resulted in decreased growth as well as enzyme synthesis. Milazzo & Fitzgerald (1967) recorded similar observations in *P. rettgeri* where maximum growth and highest enzyme activity occurred at 28°C. When temperature was raised above 29°C the arylsulfatase synthesis was inhibited significantly more than the growth.

Besides pH and temperature, the growth of bacteria in any medium is known to be influenced to a greater extent by the carbon and nitrogen sources present in it. There are some interesting observations by the effect of glucose on the arylsulfatase activity in some bacteria. Whitehead *et al.* (1952) observed that when glucose (0.5%) was added to nutrient broth, arylsulfatase activity was found to be inhibited in certain *Salmonella* and *Mycobacterium*. Similarly addition of glucose to the cultural medium resulted in complete loss of activity in *A. aerogenes* (Harada, 1957). Further it was found that glucose inhibited not only arylsulfatase activity but also growth in *P. rettgeri* (Milazzo & Fitzgerald, 1967). Identical effects of glucose on other enzyme systems have also been reported (Roberts *et al.* 1968, Liu & Zajic, 1972). Based on these findings it was thought worthwhile to study the effects of glucose on growth and arylsulfatase production in *E. coli* presently. Medium was supplemented with glucose at concentrations ranging from 0.1 to 10% and the enzyme activity was measured as described earlier. The results (Fig. 4) show that glucose at a concentration of 0.1% exerted a drastic reduction in arylsulfatase synthesis and at 0.2% level caused total loss of activity. With increasing concentrations of glucose reduction in the growth rate was also discernible, since the pH of the culture medium decreased (pH 4.2) with increasing concentrations of glucose, this might inhibit growth as well as of arylsulfatase activity/synthesis.

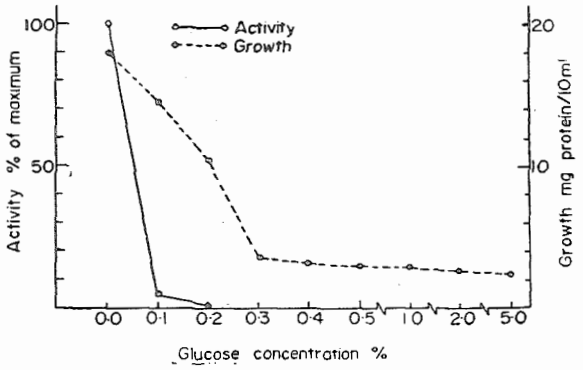

Fig. 4. Effect of glucose on the growth and arylsulfatase activity in *E. coli*. PDS–100% activity = 24.60 ug phenolphthalein liberated/mg protein/h.

Since the present strain is marinc the effect (tolerance) of sodium chloride concentration

on the growth and arylsulfatase production was also studied. For this purpose medium incorporated with varying concentrations of sodium chloride was used. The addition of sodium chloride seems to influence the growth as well as the enzyme production (Fig. 5). The enzyme activity gradually increased with an increase in sodium chloride

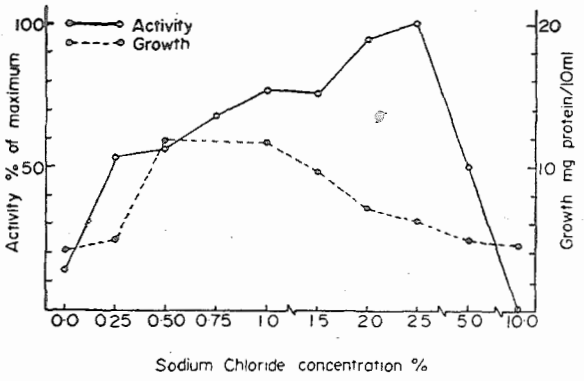

Fig. 5. Effect of sodium chloride concentration on the growth and arylfatase activity in *E. coli* PDS–100% activity = 19.87 ug phenolphthalein liberated/mg protein/h

concentration and the maximum synthesis was found at a concentration of 2.5%. However further increase resulted in decreased enzyme production and it was completely inhibited at 10% concentration. The growth was also found to be affected by increasing the concentration of sodium chloride above 1% level. Higher arylsulfatase activity at higher sodium chloride concentration may be due to the chloride (Cl^-) ions. Cl^- ions have been reported to activate arylsulfatase in the gastropod *Littorina littorea* and *Patella vulgata* and also in rat liver (Dodgson, 1949, Didgson & Spencer, 1953, Dodgson *et al.* 1953) but not the arylsulfatase of Takadiastase (Robinson et al. 1952) or of *Pseudomonas aerugionsa* (Delisle & Milazzo, 1972).

It was indicated earlier by Roy (1954) that arylsulfatases are distinguished mainly by their relative substrate affinity and behaviour towards certain inhibitors. There are reports on the effect of inhibitors and activators on arylsulfatase (Dodgson & Spencer, 1957, Roy, 1956. Shimony & Nigrelli 1972). But the effect of such compounds on the growth and production of arylsulfatases in bacteria have not so far been reported. In the present investigation, a number of compounds, known to inhibit and/or activate arylsulfatases from other sources, were tested to assess their effects on growth and arylsulfatase synthesis in *E. coli*. Sodium sulfate, sodium acetate, sodium dihydrogen phosphate and ammonium chloride were incorporated at different concentrations (6-100 x 10^{-3}M) in the medium supplemented with PDS. The results are given in Fig. 6. It was found that addition of sodium sulfate affected growth but not the arylsulfatase synthesis. Even at a concentration of 0.1 M the enzyme synthesis was apparently unaffected. However, addition of sodium dihydrogen phosphate at a concentration of 6 x 10^{-3}M enhanced synthesis of enzyme even though growth was affected to a limited extent. With further increase in concentration, growth as well as enzyme sunthesis were inhibited. When sodium acetate was added, activation of growth and enzyme synthesis were observed at a concentration of 10x10^{-3} but with further increase growth as well as enzyme synthesis decreased. Due to addition of ammonium chloride the enzyme production was effected even at a concentration of 5 x 10^{-3}M. The observed increase of enzyme activity in cultures when sodium dihydrogen phosphate was added at a concentration of 5 x 10^{-3}M may be due to the increased synthesis or due to its activation property on

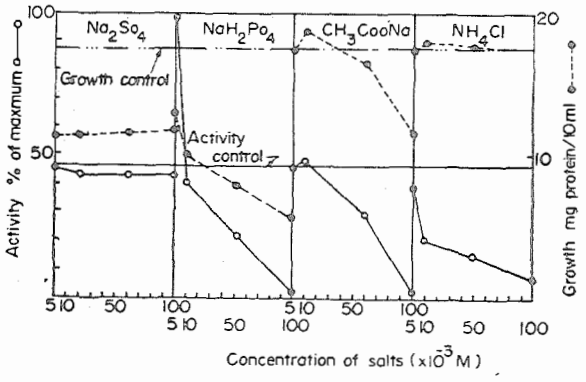

Fig. 6. Effect of added anions on the growth and arylsulfatase activity in *E. coli* PDS – 100% activity = 52.14 ug phenolphthalein liberated/mg protein/h.

the enzyme (Milazzo & Fitzgerald, 1966). Phosphate has also been reported to inhibit arylsulfatase activity in *Patella vulgata, Littorina littorea* (Dodgson & Spencer, 1953, Dodgson *et al.* 1953) and in *Balanus eburneus* (Shimony & Nigrelli, 1972). In general, concentration of phosphate ions appears to decide this effect which may be

different for different sources. Even though sulphate is known to be an inhibitor of sulfatase (Dodgson & Spencer, 1953, Harada & Spencer, 1962, Milazzo & Fitzgerald, the present study shows that it did not exert any effect on the arylsulfatase of *E. coli* even at a concentration of 0.1 M. It is interesting that in a few cases sulfate even activated arylsulfatase activity (Rammler *et al.* 1964, Delisle & Milazzo, 1972). If was found that 0.02 M sulfate repressed the sulphate of *Cephalosporium acremonium* but 5×10^{-4} sulphate depressed the sulfatase (Dannen & Carver, 1969). The observation with sodium acetate treatment indicates that concentration is an important factor since at lower concentrations it activated the enzyme synthesis and also growth but at higher concentration it inhibited both. Similar effects of activation and inhibition of arylsulfatase activity by acetate from different sources have already been reported (Dodgson, 1959, Harada & Spencer, 1964, Milazzo & Fitzgerald, 1966) Delisle & Milazzo (1972) found that acetate had no effect on the arylsulfatase of *Pseudomonas aeruginosa.* Even though ammonia may activate arylsulfatase (Dodgson, 1959 Harada & Spencer, 1964), in the present study it inhibited the synthesis since growth was almost unaffected even up to a concentration of 0.01 M. These observations suggest that concentration of a given compound is very critical in activating or inhibiting and this differs with the source of the enzyme. It is thus very difficult to generalise a compound as inhibitor or activator since this depends not only on its concentration but also on the source of the enzyme when more than one type (of arylsulfatase) is encountered in nature.

We are thankful to the authorities of Annamalai and Cochin Universities for the facility provided, to UGC, New Delhi for financial support and to Prof. N. Balakrishnan Nair, Head of the Department of Aquatic Biology and Fisheries, University of Kerala for his encouragement.

References

Adachi, T., Murooka, Y. & Harada, T. (1973) *J. Bacteriol.* **116**, 19

Buchman, R. E. & Gibbons, N. E. (1974) *Bergey's Manual of Determinative Bacteriology.* The Williams and Wilkins Co. Baltimore

Chandramohan, D., Dhevendaran, K. & Natarajan, R. (1974) *Mar. Biol.* **27**, 89

Delisle, G. J. & Milazzo, F. H. (1972) *Can J. Microbiol.* **18**, 561

Dennen, D. W. & Carver, D. D. (1969) *Can. J. Microbiol.* **15**, 175

Dhevendaran, K. (1978) *Studies in Arylsulfatase Activity of Marine Sediments. Ph.D. Thesis, Annamalai University, p.* 115

Dhevendaran, K. (1984) *Fish. Technol.* **21**, 57

Dhevendaran, K., Kannupandi, T. & Natarajan, R. (1980) *Mahasagar,* **13**, 173

Dixon, M. & Webb, E. C. (1957) in *Enzymes,* p. 81-87, Eds. (Dixon, M. and Webb, E. C.) Logmans, Green and Co. London

Dodgson, K. S. (1959) *Enzymologia* **20**, 301

Dodgson, K. S. & Spencer, B. (1953) *Biochem. J.* **43**, 444

Dodgson, K. S., Lewis, J.I.M. & Spencer, B. (1953) *Biochem. J.* **55**, 253

Dodgson, K. S., Melville, T. H., Spencer, B. & William, K. (1954) *Biochem. J.* **58**, 182

Dodgson, K. S. & Spencer, B. (1956) *Biochem J.* **62**, 30

Dodgson, K S. & Spencer, B (1957) in *Methods of Biochemical Analysis* p 211 256 Edited by Glick, D. Inter Science Publishers, N. Y.

Fowler, L. R. & Rammler, D. H. (1964) *Biochemistry* **3**, 230

Harada, T. (1957) *Bull. Agr. Chem. Soc. Japan,* **21**, 267

Harada, T. (1964) *Biochem. Biophys. Acta,* **81**, 193

Harada, T. & Spencer, B. (1964) *Biochem. J.* **93**, 373

Harada, T. & Spencer, B. (1962) *Biochem. J.* **82**, 148

Liu, F. S. & Zajic, J. (1972) *Appl. Microbial,* **23**, 667

Lowrey, O. H. Rosenbrough, Farn, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265

Milazzo, F. H. & Fitzgerald, J. W. (1966) *Can. J. Microbiol.* **12**, 735

Milazzo, F. H. & Fitzgerald, J. W. (1967) *Can. J. Microbiol.* **13**, 659

Rammler, D. H., Grado, C. & Fowler, L.R. (1964) *Biochemistry* **3**, 224

Roberts, J., Burson, G. & Hill, J. M. (1968) *J. Bacteriol.* **95**, 2117

Robinson, D., Smith, J. N., Spencer, B. & Williams, R. T. (1952) *Biochem. J.* **51**, 202

Roy, A. B. (1954) *Biochem. J.* **57**, 465

Roy, A. B. (1956) *Biochem. J.* **64**, 651

Selvakumar, N., Chandramohan, D. & Natarajan, R. (1977) *Curr. Sci.* **46**, 287

Shimony, T. & Nigrelli, R. F. (1972) *Mar. Biol.* **14**, 349

Webb, E. C. (1958) in *Enzymes* pp. 81-87 Eds. (Dixon, M. & Webb E. C.) Logmans, Green and Company, London