Carbon and Sulphur Utilization during Growth of *Pseudomonas fluorescens* on Potassium D-Glucose 6-*O*-Sulphate as the Sole Sulphur Source

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Pseudomonas fluorescens N.C.I.B. 8248, cultured on potassium D-glucose 6-[³⁵S]-*O*-sulphate as the sole sulphur source, liberated the 6-*O*-sulphate ester of D-gluconate into the culture medium. Extracts of bacteria grown under this cultural condition oxidized D-glucose 6-*O*-sulphate to yield the gluconate ester. Results suggest the involvement of a glucose dehydrogenase-like enzyme. The gluconate ester was apparently not oxidized further to any significant extent; however, it served as substrate for a desulphating enzyme found in extracts. Growth on D-glucose 6-*O*-sulphate as the sole source of sulphur was not associated with the appearance of a true glycosulphatase. Collectively, these results suggest that D-gluconate 6-*O*-sulphate, rather than the glucose ester, supplied the necessary sulphur for growth. Oxidative activities toward D-glucose 6-*O*-sulphate, D-glucose, D-gluconate 6-*O*-sulphate and D-gluconate found in extracts of *P. fluorescens* adapted to grow on D-glucose 6-*O*-sulphate as the sole source of carbon and sulphur are presented for comparative purposes.

Previous work (Fitzgerald & Dodgson, 1971) showed that *Pseudomonas fluorescens* N.C.I.B. 8248 can be adapted to grow on potassium D-glucose 6-*O*-sulphate as the sole source of carbon and sulphur. Under these circumstances the 6-*O*-sulphate ester of D-gluconate, the 3-*O*-sulphate ester of D-glycerate and traces of an unidentified sulphate ester are released into the culture medium. These and other results suggested that utilization of D-glucose 6-*O*-sulphate may occur via routes analogous to or identical with either the hexose monophosphate or Entner–Doudoroff pathways.

During studies on the capacity of a number of different unadapted bacteria to utilize D-glucose 6-*O*-sulphate, it was noted that the metabolism of the ester was simplified when it was added as the sole source of sulphur in conjunction with a more readily utilizable carbon source. The present report concerns the catabolism of D-glucose 6-*O*-sulphate by *P. fluorescens* N.C.I.B. 8248 when the organism was cultured on the ester as the sole sulphur source with glycerol as the principal carbon source.

A preliminary account of some aspects of this work has already been given (Fitzgerald & Dodgson, 1970).

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MATERIALS AND METHODS

Sulphate esters. The preparation of unlabelled potassium D-glucose 6-*O*-sulphate and dipotassium DL-glycerate 3-[³⁵S]-*O*-sulphate was described by Fitzgerald, Thomas, Tudball & Winterburn (1971). Potassium D-glucose 6-[³⁵S]-*O*-sulphate (specific radioactivity of 11.6 μ Ci/mg) was kindly supplied by Mr D. I. Bain and was freed from traces of contaminating heavy metals as described by Fitzgerald & Dodgson (1971).

Unlabelled dipotassium D-gluconate 6-*O*-sulphate was prepared by the pH-controlled bromine oxidation of potassium D-glucose 6-*O*-sulphate as described for the ³⁵S-labelled compound by Fitzgerald & Dodgson (1971). The i.r. spectrum of the unlabelled ester was identical with that of D-gluconate 6-*O*-sulphate prepared by direct sulphation of D-gluconolactone (Lloyd, Large, Tudball, Wusteman & Dodgson, 1965c). Mixtures of labelled and unlabelled ester gave material of constant specific radioactivity through six successive recrystallizations.

Culture conditions. *P. fluorescens* (National Collection of Industrial Bacteria strain 8248) was maintained by periodic subculture on tryptic soy agar slants (Difco dehydrated). Unless otherwise stated, the organism was grown on 0.08% potassium D-glucose 6-*O*-sulphate as the sole added sulphur source in a medium of the following composition: glycerol (20 g), KH₂PO₄ (3 g), K₂HPO₄ (7 g), NH₄Cl (1 g), MgCl₂·6H₂O (0.3 g), NaCl (1 g) and water (1 litre). The sulphate ester was sterilized by filtration through a Millipore membrane filter (0.22 μ m) and other components of the medium were autoclaved separately.

The pH of the complete medium was 7.0. Cells were grown at 30°C, with shaking, in 2-litre Erlenmeyer flasks containing 500 ml of medium. Inocula were prepared from 16 h nutrient broth cultures (Difco dehydrated) and the size of the inoculum was adjusted so that after inoculation the culture medium had an initial extinction (650 nm) of approx. 0.02 (Unicam SP.600).

On some occasions a mixture of D-glucose (0.048%) and K_2SO_4 (0.047%) was substituted for D-glucose 6-O-sulphate. Other experiments involved *P. fluorescens* which had been adapted to grow on D-glucose 6-O-sulphate as sole carbon and sulphur source, as described by Fitzgerald & Dodgson (1971). As a partial control for *P. fluorescens* which had been so cultured the unadapted organism was also grown on D-glucose (0.97%) and K_2SO_4 (0.94%) as sole sources of carbon and sulphur.

Determination of enzyme activities. The preparation of cell-free extracts and the determination of protein and desulphating activity toward ^{35}S -labelled sulphate esters have been described by Fitzgerald & Dodgson (1971).

Glucose dehydrogenase [glucose-(acceptor) oxidoreductase, EC 1.1.99.a] activity in cell-free extracts was determined by the 2,6-dichlorophenol-indophenol reduction method of Hauge (1966). When potassium D-glucose 6-O-sulphate replaced D-glucose as enzyme substrate the same conditions (including substrate concentration) were employed. One enzyme unit is defined as the amount of enzyme that oxidizes 1 μ mol of either D-glucose or its 6-O-sulphate ester per min. The assay conditions employed when the sulphate ester was the substrate were not necessarily optimum.

Gluconate dehydrogenase [D-gluconate-(acceptor) oxidoreductase, EC 1.1.99.3] activity in cell-free extracts was determined by the method of Wood, Fetting & Hertlein (1962). Potassium D-gluconate, dipotassium D-gluconate 6-O-sulphate and trisodium D-gluconate 6-O-phosphate were employed (under identical conditions) as assay substrates. An enzyme unit is defined as the amount of enzyme that oxidizes 1 μ mol of substrate/h. The assay conditions are not necessarily optimum for the sulphate or phosphate esters.

With all the enzymes mentioned above, enzyme activity was linear with respect to the time of incubation. All oxidative and desulphating activities are expressed as the number of enzyme units/mg of protein.

Measurement of concentrations of D-glucose 6-O-sulphate and metabolite A in culture media. The disappearance of D-glucose 6-O-sulphate from culture media and the release of metabolite A into media during growth was followed by including D-glucose 6- ^{35}S -O-sulphate in the media. Culture samples, taken at time-intervals during growth, were clarified by centrifugation followed by Millipore filtration (0.22 μ m). Samples (20 μ l) of clear filtrates were spotted on Whatman no. 1 paper and subjected to electrophoresis in 0.1 M-sodium acetate-acetic acid buffer, pH 4.5, at 22 V/cm for 1.5 h. Radioactive areas were located and their radioactivities measured as described by Dodgson, Lloyd & Tudball (1961).

EXPERIMENTAL AND RESULTS

Production and identification of metabolite A. *P. fluorescens* was grown at 30°C in 20 ml of medium containing 32 mM-potassium D-glucose 6- ^{35}S -O-

sulphate (mixture of 5 mM labelled and 27 mM unlabelled) as sulphur source with 2% (w/v) glycerol as the principal carbon source. Fig. 1 shows the relationship between culture-age, growth and the progressive liberation of a ^{35}S -labelled intermediate into the culture medium. At the termination of growth, 12.2% of the initial total radioactivity had disappeared from the medium, 57.4% was present as starting material and the intermediate (metabolite A) made up the remainder (30.4%).

Metabolite A was separated from unchanged D-glucose 6- ^{35}S -O-sulphate by thick-layer chromatography on silica gel G. Ethyl acetate-formamide-pyridine (6:4:1, by vol.) was employed as developing solvent and the elution of the metabolite from the gel was as described for the isolation of D-glycerate 3-O-sulphate by Fitzgerald & Dodgson (1971). The separated metabolite migrated as a single component during paper electrophoresis and paper chromatography (in six different solvents; see Fitzgerald & Dodgson, 1971) with mobilities identical with those for authentic D-gluconate 6- ^{35}S -O-sulphate. Further confirmation of identity was obtained by mixing it with an excess of chemically synthesized unlabelled D-gluconate 6-O-sulphate and recrystallizing the whole six times. Material of constant specific radioactivity was obtained after the first crystallization.

Oxidation of potassium D-glucose 6-O-sulphate by extracts of *P. fluorescens*. Bacteria were grown with D-glucose 6-O-sulphate as sole source of sulphur and

Fig. 1. Liberation of metabolite A by *P. fluorescens* during growth on D-glucose 6- ^{35}S -O-sulphate as the sole source of sulphur with glycerol as the principal carbon source. ●, Culture extinction; ○, metabolite A.

glycerol as major carbon source. Extracts (5ml containing 50–75mg of protein) prepared from stationary-phase cells were incubated with 5ml of 20mM-D-glucose 6-[35 S]-O-sulphate in 50mM-glycylglycine-NaOH buffer, pH 7.5, for 8 h at 37°C. Paper electrophoresis of a sample of the reaction mixture after incubation showed the presence of an additional radioactive component. The remainder of the reaction mixture was treated with an equal volume of 8% (w/v) trichloroacetic acid and precipitated protein was removed by centrifugation. The clear supernatant was extracted eight times with an equal volume of diethyl ether and neutralized with 0.05M-potassium hydroxide. The neutral supernatant was then concentrated by freeze-drying and subjected to thick-layer chromatography on silica gel G to separate the reaction product from unchanged D-glucose 6-[35 S]-O-sulphate (see the preceding section). The product was eluted from the gel and identified as D-gluconate 6-O-sulphate by chromatographic criteria and also by isotope-dilution analysis.

Although these results established that D-glucose 6-O-sulphate could be oxidized to the corresponding gluconate 6-O-sulphate attempts to show that the process was analogous to that involved in the oxidation of D-glucose 6-O-phosphate were completely unsuccessful. Thus addition of NAD $^{+}$ or NADP $^{+}$ to cell extracts producing D-gluconate

6-[35 S]-O-sulphate from D-glucose 6-[35 S]-O-sulphate did not increase the amounts being produced. Further, extracts of cells harvested at different time-intervals during growth on D-glucose 6-O-sulphate did not reduce either NAD $^{+}$ or NADP $^{+}$ when the sulphate ester was substituted for D-glucose 6-phosphate in the procedure (Kuby & Noltmann, 1966) normally employed for the assay of D-glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate-NADP $^{+}$ oxidoreductase, EC 1.1.1.49).

Extracts were next examined for their ability to oxidize D-glucose 6-O-sulphate by using the assay method developed for glucose dehydrogenase (EC 1.1.99.a; see Barman, 1969). The organism was grown under four different cultural conditions, namely: D-glucose 6-O-sulphate as sole source of carbon and sulphur; D-glucose 6-O-sulphate as sole sulphur source and glycerol as carbon source; D-glucose as carbon source and potassium sulphate as sole sulphur source; D-glucose and glycerol as carbon sources and potassium sulphate as sulphur source. Fig. 2 shows the dehydrogenase activities exhibited towards both D-glucose and its 6-O-sulphate ester by extracts of cells removed from the various cultures at different time-intervals. Dehydrogenase activity towards both substrates was always maximal in extracts prepared from stationary-phase cells and D-glucose 6-O-sulphate was oxidized in all cases. When the sulphate ester was

Fig. 2. Relationship between culture age and oxidation of D-glucose (○) and D-glucose 6-O-sulphate (●) by extracts of *P. fluorescens* grown on (a) 1.6% (w/v) D-glucose 6-O-sulphate as sole source of carbon and sulphur at 37°C, (b) 0.08% D-glucose 6-O-sulphate as sole sulphur source and 2.0% (w/v) glycerol as carbon source at 30°C, (c) 0.97% D-glucose as carbon source and 0.94% K $_2$ SO $_4$ as sulphur source at 30°C and (d) 0.048% D-glucose and 2.0% (w/v) glycerol as carbon source and 0.047% K $_2$ SO $_4$ as sulphur source at 30°C. Growth curves for each cultural condition are omitted for the sake of clarity and replaced by markings indicating the various phases of growth (L, lag; E, exponential; S, stationary). Oxidation was measured by the glucose dehydrogenase assay; see the text for details.

the sole sulphur source, glucose dehydrogenase activities towards both D-glucose and its sulphate ester (Fig. 2*b*) compared favourably with those obtained (Fig. 2*d*) when glycerol, glucose and potassium sulphate were supplying carbon and sulphur (ratio of peak specific activities of D-glucose 6-*O*-sulphate and D-glucose: for Fig. 2*b*, 0.22; for Fig. 2*d*, 0.16). Similarly, activities towards the two substrates when the organism was grown on D-glucose and potassium sulphate as sole carbon and sulphur sources (Fig. 2*c*) compared favourably with those obtained (Fig. 2*a*) when the sole carbon and sulphur source was D-glucose 6-*O*-sulphate (ratio of peak specific activities: for Fig. 2*c*, 0.52; for Fig. 2*a*, 0.60).

Irrespective of the culture conditions *P. fluorescens* was therefore capable of oxidizing D-glucose 6-*O*-sulphate and this was further substantiated when extracts from cells grown on D-glucose and potassium sulphate were incubated with the ³⁵S-labelled sulphate ester. Under these circumstances the ester was converted into a labelled metabolite that was identical with D-gluconate 6-*O*-sulphate according to electrophoretic and chromatographic criteria. As before, maximum formation of the labelled metabolite occurred with extracts prepared from stationary-phase cells.

Collectively these results suggest that the oxidation of D-glucose 6-*O*-sulphate to D-gluconate 6-*O*-sulphate is mediated by an enzyme that

resembles or is identical in type with glucose dehydrogenase, but proof of this must await purification of the enzyme. Meanwhile, some preliminary evidence suggests that the oxidation may also be achieved by a mechanism analogous to the conversion of D-glucose into D-gluconate by fungal glucose oxidase (β -D-glucose-oxygen oxidoreductase, EC 1.1.3.4; see Pazur, 1966) or the oxidation of D-galactose by fungal galactose oxidase (D-galactose-oxygen oxidoreductase, EC 1.1.3.9). Extracts prepared from cells grown on D-glucose 6-*O*-sulphate (either as sole sulphur source or as carbon and sulphur source) utilized the ester to oxidize *o*-dianisidine in the presence of added horseradish peroxidase in a manner analogous to that operating in assay methods for D-glucose oxidase and D-galactose oxidase (assay conditions were those employed for the assay of the latter enzyme; see Amaral, Kelly-Falcoz & Horecker, 1966). In contrast with the results obtained with the glucose dehydrogenase assay (see Fig. 2*b*), maximum activity as indicated by the oxidase assay method was found in early exponential-growth-phase cells, suggesting that both types of enzyme system may be involved in the oxidation of D-glucose 6-*O*-sulphate by *P. fluorescens* but at different stages during growth.

Oxidation of D-gluconate 6-O-sulphate by P. fluorescens. The capacity of *P. fluorescens* to oxidize D-gluconate 6-*O*-sulphate was tested by

Fig. 3. Relationship between culture age and oxidation of D-gluconate (O) and D-gluconate 6-*O*-sulphate (●) by extracts of *P. fluorescens* grown on (a) 0.08% D-glucose 6-*O*-sulphate as sole sulphur source and 2.0% (w/v) glycerol as carbon source at 30°C and (b) 1.6% (w/v) D-glucose 6-*O*-sulphate as sole source of carbon and sulphur at 37°C. Growth curves for each cultural condition are omitted for the sake of clarity and replaced by markings indicating the various phases of growth (L, lag; E, exponential; S, stationary). Oxidation was measured by the gluconate dehydrogenase assay; see the text for details.

assaying extracts for gluconate dehydrogenase activity with the sulphate ester as assay substrate. Fig. 3(a) shows the relationship between culture age and dehydrogenase activity towards this substrate and towards D-gluconate in extracts prepared from cells grown on D-glucose 6-O-sulphate as the sole sulphur source. Results of similar experiments with cells grown on D-glucose 6-O-sulphate as the sole source of carbon and sulphur are given for comparative purposes (Fig. 3b). In contrast with the glucose dehydrogenase activity in similar extracts (cf. Figs. 2 and 3), maximum gluconate dehydrogenase activity occurred in cells of the early to mid-exponential growth phase. Fig. 3(a) further shows that activity towards D-gluconate 6-O-sulphate in cells grown on D-glucose 6-O-sulphate as sole sulphur source is really quite low and this presumably accounts for the release of considerable amounts of the gluconate sulphate into the culture medium (see Fig. 1). In contrast, when D-glucose 6-O-sulphate was acting as sole carbon and sulphur source, the rate of oxidation of D-gluconate 6-O-sulphate was much higher and in excess of that exhibited towards D-gluconate [ratio of peak specific activities of D-gluconate 6-O-sulphate and D-gluconate is 1.2 (see Fig. 3b); cf. Fig. 3(a) where the ratio is 0.11]. Under these latter cultural conditions relatively little D-gluconate 6-O-sulphate appears in the culture medium (approx. 4% of the initial concentration of D-glucose 6-O-sulphate; see Fitzgerald & Dodgson, 1970, 1971). Trisodium 6-phosphogluconate was not oxidized by extracts of cells grown under either cultural condition.

Extracts of cells grown on D-glucose and potassium sulphate or on D-glucose, glycerol and potassium sulphate were able to oxidize D-gluconate (maximum specific activities are 3.6 and 3.3 enzyme units/mg of protein respectively). However, they did not attack the corresponding sulphate ester. It is worth noting that only very small amounts of D-glucose (0.048%) are required in the medium for the cell extracts to contain gluconate dehydrogenase of high specific activity (3.3 enzyme units/mg of protein). When D-glucose 6-O-sulphate (0.08%) replaced the parent monosaccharide in the culture medium the synthesis of a dehydrogenase active towards D-gluconate was considerably decreased (0.66 enzyme unit/mg of protein; Fig. 3a). Similar consideration applied when the sulphate ester replaced D-glucose at a concentration of 1.6% (0.42 enzyme unit/mg of protein for cells grown as described for Fig. 3b, compared with 3.6 units/mg of protein for cells grown on D-glucose and potassium sulphate).

Desulphation of D-gluconate 6-O-sulphate by *P. fluorescens*. When D-gluconate 6[³⁵S]-O-sulphate (specific radioactivity approx. 0.14 μ Ci/mg) was substituted for D-glucose 6[³⁵S]-O-sulphate as sole

sulphur source the maximum culture extinction at 650nm was higher (3.6 as opposed to 2.7) and, in terms of disappearance of radioactivity from the medium, the gluconate ester was utilized to a greater extent as sulphur source (29.1% disappeared compared with 12.2%). The remainder (70.9%) of the initial radioactivity was still present in the medium as D-gluconate 6[³⁵S]-O-sulphate. No labelled metabolites could be detected in the medium even when samples of the latter were concentrated 100-fold.

Attempts to detect a glycosulphatase (sugar sulphate sulphohydrolase, EC 3.1.6.3) in extracts of cells grown on D-glucose 6-O-sulphate as sole sulphur source were unsuccessful. Fitzgerald & Dodgson (1971) obtained similar results with extracts from cells grown on the ester as sole carbon

Fig. 4. (a) Relationship between culture extinction and utilization of D-glucose 6[³⁵S]-O-sulphate during growth of *P. fluorescens* at 30°C on 0.08% D-glucose 6-O-sulphate + 5mm-D-glucose 6[³⁵S]-O-sulphate as the sole sulphur source with 2.0% (w/v) glycerol as carbon source. ○, Culture extinction; ●, utilization of D-glucose 6[³⁵S]-O-sulphate; □, culture extinction of the inoculated medium in the absence of D-glucose 6-O-sulphate. (b) Relationship between culture age and desulphating activity in *P. fluorescens* grown as for (a). Δ, Desulphation of D-gluconate 6[³⁵S]-O-sulphate. Dialysed extracts were incubated with 20 mM substrate at 30°C for 4 h in 0.05 M-glycylglycine-NaOH buffer, pH 7.5. ----, Culture pH.

and sulphur source. It therefore appeared probable that D-gluconate 6-O-sulphate rather than the glucose analogue supplied the sulphur for growth on D-glucose 6-O-sulphate. To test this proposition extracts were examined for their ability to liberate inorganic $^{35}\text{SO}_4^{2-}$ ions from D-gluconate 6- ^{35}S -O-sulphate. Maximum desulphation (Fig. 4) occurred just after the onset of exponential growth and linear utilization of D-glucose 6-O-sulphate. No ^{35}S -labelled product other than inorganic $^{35}\text{SO}_4^{2-}$ ions was formed, indicating that the gluconate sulphate was not first converted into another sulphated compound. It also follows that the liberated inorganic $^{35}\text{SO}_4^{2-}$ ions were not incorporated into other compounds during the assay procedure. Extracts were not capable of hydrolysing either D-glucose 6- ^{35}S -O-sulphate or DL-glycerate 3- ^{35}S -O-sulphate under optimum conditions for the liberation of inorganic $^{35}\text{SO}_4^{2-}$ ions established for the glycosulphatase of *Trichoderma viride* (Lloyd, Large, Davies, Olavesen & Dodgson, 1968; see also Fitzgerald & Dodgson, 1971). It should be noted that some growth occurred (Fig. 4) when the organism was cultivated in the same medium without D-glucose 6-O-sulphate. This may probably be attributed to the presence of traces of sulphur-containing materials in the washed inoculum and in the reagents constituting the medium.

DISCUSSION

The utilization of D-glucose 6-O-sulphate by *P. fluorescens* when the ester is supplied as sole sulphur source differs markedly from that exhibited when the ester has the additional role of serving as sole source of carbon also. In the first situation some of the ester is oxidized to D-gluconate 6-O-sulphate, which may then act as a source of sulphur for growth, presumably after the intervention of the desulphating enzyme system. Excess of gluconate sulphate accumulates in the medium. In the second situation both formation and subsequent oxidation of D-gluconate 6-O-sulphate occurs. Relatively small amounts of gluconate sulphate appear in the medium and the major portion is catabolized to D-glycerate 3-O-sulphate (Fitzgerald & Dodgson, 1971) and (presumably) to pyruvate. The former appears in large quantities in the medium and probably also serves as the principal source of sulphur after the intervention of a cell-bound desulphating enzyme active towards this ester. Carbon and energy for growth presumably come from the pyruvate.

Under either cultural circumstance, the oxidation of D-glucose 6-O-sulphate is apparently similar to the oxidation of D-glucose, suggesting attack by the same type of enzyme (glucose dehydrogenase).

The capacity of glucose dehydrogenase to oxidize D-glucose 6-O-sulphate does not appear to have been reported. However, partially purified enzymes from many *Pseudomonas* species have been shown to oxidize a wide range of aldoses including mono- and di-saccharides (Hauge, 1966). Further, in investigations on the ability of 2-deoxy-D-glucose to act as an inhibitor of glucose oxidation in *Pseudomonas aeruginosa*, Williams & Eagon (1959) have noted that the inhibitor is itself oxidized to 2-deoxy-D-gluconate. Two separate enzymes are able to catalyse this reaction, one indistinguishable from glucose dehydrogenase and the other being able to attack only 2-deoxy-D-gluconate. As in the present studies oxidative activity towards the analogue does not require the latter to be present in the culture medium (Williams & Eagon, 1959). It is not clear whether growth on 2-deoxy-D-glucose leads to the appearance of 2-deoxy-D-gluconate in the culture medium but there have been several reports of the accumulation of D-gluconate when various Pseudomonads were grown on D-glucose (Gunsalus, Horecker & Wood, 1955; De Ley, 1960). Possibly 2-deoxy-D-glucose and D-glucose 6-O-sulphate are treated in the same manner as the parent monosaccharide. However, this generalization may not apply in all cases and the same strain of *P. aeruginosa* is known to transport D-glucose and 2-deoxy-D-glucose by different mechanisms when cells are grown on D-glucose. Data suggest active transport by induction for the parent monosaccharide and free diffusion for the analogue (R. G. Eagon, unpublished results).

It is noteworthy that the mould *T. viride* releases D-gluconate 6-O-sulphate into the medium when grown on D-glucose 6-O-sulphate. This transformation can be accomplished by cell-free extracts of the organism and the oxidation is NADP^+ -dependent. In this case there are indications that D-glucose 6-phosphate dehydrogenase or a closely related enzyme is the responsible agent (Lloyd *et al.* 1965a; Lloyd, Large, Tudball & Dodgson, 1965b; Lloyd *et al.* 1965c).

Pseudomonads apparently differ in their ability to oxidize D-gluconate and in many species, including *P. fluorescens*, 2-keto-D-gluconate (D-arabino-2-hexulosonate) may be released into the culture medium (De Ley, 1960). Of a number of species tested, only *Pseudomonas ovalis* formed D-gluconate in considerable amounts (Lockwood, Tabenkin & Ward, 1941). This finding may reflect the absence of a dehydrogenase capable of oxidizing D-gluconate further. When *P. fluorescens* N.C.I.B. 8248 was adapted to growth on D-glucose 6-O-sulphate as sole carbon and sulphur source (see Fitzgerald & Dodgson, 1971) cell-free extracts were able to oxidize D-gluconate 6-O-sulphate as well as D-gluconate. Dehydrogenase activity towards the

sulphate ester could not be detected in extracts of cells grown on D-glucose or glycerol even though such extracts oxidized D-gluconate. The precise significance of this is not yet clear although culture age-enzyme activity profiles for the oxidation of each compound are virtually identical. This may suggest the induction, by the adapted strain, of a dehydrogenase active towards both sulphate ester and parent compound. However, the possibility of the synthesis of an additional dehydrogenase without dual substrate specificity cannot be excluded. Certainly there are indications that purified D-gluconate dehydrogenase from *P. fluorescens* exhibits high specificity for D-gluconate (Wood *et al.* 1962).

The enzyme responsible for the desulphation of D-gluconate 6-[³⁵S]-O-sulphate is of the rare type I variety according to Gale's classification of microbial enzymes (Gale, 1943, 1952). This property also characterizes desulphating activity toward the same substrate found in extracts from *P. fluorescens* N.C.I.B. 8248 grown under a number of other cultural conditions (see Fitzgerald & Dodgson, 1971). It is therefore probable that the enzyme is constitutive and a similar situation may be argued for the dehydrogenase responsible for the formation of D-gluconate 6-O-sulphate. One might therefore expect that a wide range of Pseudomonads and related bacteria (see De Ley, 1960) would be able to utilize D-glucose 6-O-sulphate as a source of sulphur in the presence of a suitable carbon source. Preliminary results with several species of *Pseudomonas* support this view.

Finally, the contrast between sulphatase activity in *T. viride* and in *P. fluorescens* is noteworthy. The enzyme developed in the former organism in response to growth on D-glucose 6-O-sulphate appears to be a true glycosulphatase acting on the 6-O-sulphate esters of D-glucose and D-galactose but not on D-gluconate 6-O-sulphate (Lloyd *et al.* 1965c, 1968). On the other hand, desulphating activity appearing in *P. fluorescens* in any of the circumstances yet tested is without effect on D-glucose 6-O-sulphate.

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REFERENCES

- Amaral, D., Kelly-Falcoz, F. & Horecker, B. L. (1966). In *Methods in Enzymology*, vol. 9, p. 87. Ed. by Wood, W. A. New York: Academic Press Inc.
- Barman, T. E. (1969). *Enzyme Handbook*, vol. 1, p. 122. Berlin: Springer-Verlag.
- De Ley, J. (1960). *J. appl. Bact.* **23**, 400.
- Dodgson, K. S., Lloyd, A. G. & Tudball, N. (1961). *Biochem. J.* **79**, 111.
- Fitzgerald, J. W. & Dodgson, K. S. (1970). *Biochem. J.* **119**, 30p.
- Fitzgerald, J. W. & Dodgson, K. S. (1971). *Biochem. J.* **121**, 521.
- Fitzgerald, J. W., Thomas, P., Tudball, N. & Winterburn, P. J. (1971). *Biochem. J.* **121**, 529.
- Gale, E. F. (1943). *Bact. Rev.* **7**, 139.
- Gale, E. F. (1952). *The Chemical Activities of Bacteria*, 3rd ed., p. 78. London: University Tutorial Press.
- Gunsalus, I. C., Horecker, B. L. & Wood, W. A. (1955). *Bact. Rev.* **19**, 101.
- Hauge, J. G. (1966). In *Methods in Enzymology*, vol. 9, p. 92. Ed. by Wood, W. A. New York: Academic Press Inc.
- Kuby, S. A. & Noltmann, E. A. (1966). In *Methods in Enzymology*, vol. 9, p. 116. Ed. by Wood, W. A. New York: Academic Press Inc.
- Lloyd, A. G., Large, P. J., Davies, M., Olavesen, A. H. & Dodgson, K. S. (1968). *Biochem. J.* **108**, 393.
- Lloyd, A. G., Large, P. J., Tudball, N., Davies, M., Olavesen, A. H. & Dodgson, K. S. (1965a). *Biochem. J.* **97**, 41p.
- Lloyd, A. G., Large, P. J., Tudball, N. & Dodgson, K. S. (1965b). *Biochem. J.* **97**, 42p.
- Lloyd, A. G., Large, P. J., Tudball, N., Wusteman, F. S. & Dodgson, K. S. (1965c). *Biochem. J.* **97**, 43p.
- Lockwood, L. B., Tabenkin, B. & Ward, G. E. (1941). *J. Bact.* **42**, 51.
- Pazur, J. H. (1966). In *Methods in Enzymology*, vol. 9, p. 82. Ed. by Wood, W. A. New York: Academic Press Inc.
- Williams, A. K. & Eagon, R. G. (1959). *J. Bact.* **77**, 167.
- Wood, W. A., Fetting, R. A. & Hertlein, B. C. (1962). In *Methods in Enzymology*, vol. 5, p. 287. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.