Preliminary Observations on Alcohol Dehydrogenases in *Comamonas terrigena* that exhibit Stereospecificity towards Secondary Alcohols

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Extracts of the cells of *Comamonas terrigena*, grown under a variety of different conditions, contain two distinct, constitutive, NAD-dependent alcohol dehydrogenase enzymes that can be separated by polyacrylamide-gel electrophoresis. One of the enzymes exhibits activity towards D-alkan-2-ols and primary alcohols and the other is active towards L-alkan-2-ols, symmetrical secondary alcohols and probably other positional isomers of secondary alcohols of the L-configuration. Methods for the individual assay of the two enzymes have been developed and have been used to define some of their general properties. Most of the substrates for these enzymes would not support growth of *C. terrigena* under the experimental conditions used and were relatively poorly oxidized by resting cell suspensions.

The soil bacterium *Pseudomonas* C12B, which degrades alkyl sulphate detergents, produces two primary and three secondary alkylsulphohydrolases and can utilize primary alcohols as a source of carbon and energy (Dodgson *et al.*, 1974; Payne, 1963). The bacterium exhibits NAD-dependent alcohol dehydrogenase activity towards primary and secondary alcohols (Williams *et al.*, 1966) and it seems probable, from studies with sodium dodecyl sulphate and dodecanol, that the micro-organism degrades primary alkyl sulphates via hydrolysis of the ester bond and oxidation of the liberated primary alcohol by an alcohol dehydrogenase (Williams & Payne, 1964). Other work on a different *Pseudomonas* species has shown that secondary alkyl sulphates can be completely oxidized to CO₂, H₂O and sulphate. Enzymic desulphation apparently precedes the initial oxidation of the liberated secondary alcohol to the corresponding ketone (Lijmbach & Brinkhuis, 1973). *Comamonas terrigena*, a co-isolate of *Pseudomonas* C12B, can also degrade some secondary alkyl sulphates and has been shown to produce two alkylsulphohydrolases. One of the enzymes, designated as CS1, is specific for L-alkan-2-yl sulphates and the other enzyme, designated as CS2, is specific for the corresponding D-isomers (Matcham *et al.*, 1977a). The hydrolysis of alkan-2-yl sulphate stereoisomers by these enzymes involves rupture of the C–O–S

ester linkage on the alcohol side of the linkage (i.e. C–O bond rupture) and is accompanied by an inversion of configuration (Bartholomew *et al.*, 1977). These observations suggested that it would be of interest to investigate the further metabolism of the optically active alcohols liberated as a result of stereospecific sulphohydrolase action, and the present paper describes preliminary studies on alcohol dehydrogenase activity in *C. terrigena*, including the presence of a novel L-alcohol dehydrogenase.

Materials and Methods

Chemicals

Resolved stereoisomers of butan-2-ol and octan-2-ol were obtained from Fluka AG, Buchs SG, Switzerland and the Aldrich Chemical Co., Milwaukee, WI, U.S.A., respectively. Other alcohols were purchased from the Aldrich Chemical Co., K and K Laboratories, Plainview, NY, U.S.A. or Eastman Organic Chemicals, Rochester, NY, U.S.A. Octyl sulphate was prepared by the method of Dodgson *et al.* (1974) and secondary alkyl sulphates were prepared using the pyridine/SO₃ reagent as described by Matcham & Dodgson (1977). NAD⁺ and NADP⁺ were obtained from Boehringer Mannheim GmbH, W. Germany. All other chemicals were the purest available from the Sigma (London) Chemical Co., or BDH Chemicals, both of Poole, Dorset, U.K.

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Bacterium and culture conditions

The origin and tentative classification of *C. terrigena* has been previously described (Fitzgerald *et al.*, 1975). Cells grown to early stationary phase in nutrient broth with shaking at 30°C were used for experiments in which alcohol dehydrogenase activities were separated by electrophoresis on polyacrylamide gels. For other work the bacterium was grown in basal salts medium (Fitzgerald *et al.*, 1975) with the addition of NaCl (0.5 g/l) and Na₂SO₄ (0.14 g/l) and a carbon source of form and concentration specified for each experiment. All components were sterilized by autoclaving except for alcohols, which were added later. On these occasions the media were incubated as above for at least five days prior to inoculation in order to ensure that no microbial contamination had occurred. Cells to be harvested were grown in batches of 350 ml of culture medium inoculated with a late exponential phase culture (1 ml) of *C. terrigena* growing in 1% (w/v) sodium pyruvate in basal salts medium. For growth experiments 100 ml portions of culture medium were inoculated in the same manner except that the inoculum was grown on 0.1% (w/v) sodium pyruvate.

Preparation of crude cell extracts

For polyacrylamide-gel electrophoresis experiments cell extracts were prepared as described by Fitzgerald & Payne (1972) except that the dialysis step was omitted. For all other experiments the extracts were prepared in the same manner but substituting 20 mM-NaH₂PO₄/Na₂HPO₄ buffer, pH 7.5, for the 10 mM-Tris/HCl buffer originally used.

Polyacrylamide-gel electrophoresis and location of alcohol dehydrogenase activity in gels

Crude cell extracts were subjected to polyacrylamide-gel electrophoresis as described by Payne & Painter (1971) with the addition of pre-electrophoresis of gels at 3 mA/gel for 2 h in the presence of gel buffer. Alcohol dehydrogenase activity was located in gels using the method of Grell *et al.* (1965), except that the stain was prepared by mixing equal volumes of a well-shaken suspension of 40 µmol of alcohol/ml of H₂O, and a solution containing NAD⁺ (3.0 g/l), phenazine methosulphate (0.1 g/l) and Nitro Blue Tetrazolium (1.0 g/l) in 100 mM-Tris/HCl buffer, pH 8.5. The 'nothing dehydrogenase' detected in polyacrylamide gels by Payne & Painter (1971) was not observed in gels prepared during the present study, but gels incubated in stain in the absence of alcohol developed a general non-specific colouration. This was eliminated by soaking gels in cold stain-buffer for 30 min before incubating in stain.

Alcohol dehydrogenase assay and protein determination

Alcohol dehydrogenase activity at 30°C was measured spectrophotometrically by following the D-octan-2-ol, octan-1-ol or L-octan-2-ol-dependent reduction of NAD⁺ at 340 nm using a Beckman spectrophotometer (model 25). Incubation mixtures contained 6 µmol of alcohol/ml and 1 mM-NAD⁺ in 50 mM-NaH₂PO₄/Na₂HPO₄ buffer, pH 8.5, in a final assay volume of 1.4 ml. Under these conditions each alcohol was present in greater quantity than that equivalent to its maximum solubility. In order to achieve the maximum solubilization of alcohol in the assay mixture, phosphate buffer containing the alcohol was vigorously shaken before adding 1 ml to the cuvette. NAD⁺ was then added (in 200 µl of buffer) and, after temperature equilibration, the reaction was started by adding 200 µl of pre-warmed enzyme solution containing sufficient enzyme to give an initial rate of increase in absorbance of 0.15–0.25 units/min. Suitable control determinations were made in which the alcohol substrate was omitted. A unit of enzyme activity is defined as the amount of enzyme that reduces NAD⁺ at a rate of 1 µmol/min.

Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin (fraction V) as standard.

Preparation of resting cell suspensions and measurement of oxygen consumption

Cells grown on defined media were prepared as described by Fitzgerald & Payne (1972) so that the final cell concentration was approx. 20 mg dry wt./ml. The rates of oxygen consumption by cell suspensions in the presence of various alcohols in basal salts medium at 30°C were measured using an oxygen electrode (Rank Brothers, Bottisham, Cambs., U.K.). All alcohols were present at concentrations below their maximum solubility.

Results and Discussion

Polyacrylamide-gel electrophoresis and assay of alcohol dehydrogenase activity

Gels stained for alcohol dehydrogenase activity with racemic alkan-2-ols developed two bands of activity, which migrated anodically. The bands appeared with all racemic alcohols of chain-lengths from four to twelve carbons (see Fig. 1a for example). However, with D-octan-2-ol only the slower moving band was present (Fig. 1b) whilst L-octan-2-ol gave only the faster moving band (Fig. 1c). A similar distinction was noted for the stereoisomers of butan-2-ol. In the presence of primary alcohols of chain-lengths from two to twelve carbons a single band of activity appeared corresponding in

Fig. 1. Polyacrylamide gels stained for alcohol dehydrogenase activity towards various alcohols

Gels were stained as described in the text, using (a) DL-heptan-2-ol; (b) D-octan-2-ol; (c) L-octan-2-ol; (d) heptan-1-ol; (e) DL-nonan-3-ol.

position to the D-alkan-2-ol dehydrogenase. A single band also appeared when D-octan-2-ol and nonan-1-ol were present together in the stain, suggesting that a single enzyme was responsible for both activities. After prolonged incubation (6–12 h) in stain containing a primary alcohol, an additional very weak dehydrogenase band appeared, corresponding in position to the L-alkan-2-ol dehydrogenase (Fig. 1d). This same single band also appeared when racemic and symmetrical secondary alcohols with the hydroxyl group at positions other than C-2 were used, e.g. decan-3-ol, heptan-4-ol and nonan-5-ol (see Fig. 1e for example) although, judged by the time taken for the appearance of the band, these were less effective substrates than the L-alkan-2-ols. There was general staining of the lower part of the gel but no specific staining with primary or secondary alcohols when NAD^+ was replaced as co-factor by NADP^+ .

Subsequently, D-alkan-2-ol dehydrogenase activity in cell extracts was assayed using D-octan-2-ol or octan-1-ol as substrate, whilst L-octan-2-ol was selected as substrate for the L-alkan-2-ol dehydrogenase. The enzyme activities observed in extracts were proportional to protein concentration up to the highest concentration tested (1 mg/ml of incubation mixture). Negligible endogenous dehydrogenase activity was observed in control determinations. Maximum rate of reduction of NAD^+ was obtained when alcohol substrates were present at a concentration of 6 $\mu\text{mol/ml}$ (Fig. 2a) and when NAD^+ was present at a concentration of 1.0 mM (Fig. 2b).

Fig. 2. Effect of concentration of substrates on the alcohol dehydrogenase activity in cell-free extracts of *C. terrigena*

The effects of (a) varying the alcohol concentration at an NAD^+ concentration of 1 mM and (b) varying the NAD^+ concentration at a constant alcohol content of 6 $\mu\text{mol/ml}$ of incubation mixture are shown. ■, Octan-1-ol; □, D-octan-2-ol; △, L-octan-2-ol. See the text for further details.

With respect to the alcohol substrates, all three dehydrogenase activities exhibited simple Michaelis-Menten kinetics up to saturating concentrations of the alcohols, at which point activities became constant. Double reciprocal plots gave apparent K_m values of 3.6 mM, 2.5 mM and 2.1 mM for D-octan-2-ol, octan-1-ol and L-octan-2-ol respectively. Double reciprocal plots for the data obtained for NAD^+ concentration gave apparent K_m values of 0.17 mM, 0.14 mM and 0.052 mM when D-octan-2-ol, octan-1-ol and L-octan-2-ol respectively, were employed as alcohol substrates.

The pH-enzyme activity profiles for the three alcohol dehydrogenase activities are shown in Fig. 3. Activities towards D-octan-2-ol and octan-1-ol had similar profiles with a maximum at pH 8.5, whilst L-octan-2-ol dehydrogenase activity gave a different profile with maximum activity being constant between pH 7.5 and 9.7. From Figs. 2 and 3 it can be deduced that the ratio of activities towards octan-1-ol and D-octan-2-ol is remarkably constant at a value of about 1.2 and this provides additional support for the view that a single enzyme was responsible for both activities.

Both alcohol dehydrogenases were detected only

Fig. 3. Effect of pH on alcohol dehydrogenase activity in cell-free extracts of *C. terrigena*

Enzyme activity was measured towards ■, octan-1-ol; □, D-octan-2-ol; and △, L-octan-2-ol as described in the text, except that the buffers used were 50 mM-Tris/HCl for pH 6.30, 7.45 and 8.55; 50 mM- $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ for pH 6.05, 7.00 and 7.70; 50 mM-glycine/NaOH for pH 8.70 and 9.70. Incubation mixtures contained 0.14 mg of protein/ml for assays with octan-1-ol or D-octan-2-ol, and 0.28 mg of protein/ml with L-octan-2-ol.

in cell-free extracts and not in the residual cell debris. Both enzymes were also present with undiminished activity in the supernatant of a cell-extract that had been centrifuged for 1 h at 120 000 g_{av} , and no activity was detected in the pellet, suggesting that the activities were either not membrane bound or were readily released from association with membranes during the preparative procedures employed.

No alcohol dehydrogenase activities were detected in cell extracts when NAD^+ was replaced by NADP^+ .

Appearance of alcohol dehydrogenase activities during batch culture

C. terrigena was grown in batch culture on nutrient broth, on basal salts containing 1% (w/v) sodium pyruvate or on basal salts containing sodium pyruvate and 2 mM-racemic octan-2-ol. Cells were harvested at intervals and extracts were assayed for alcohol dehydrogenase activities and protein in order to assess the specific activities of the enzymes. Growth on all three media gave essentially similar

results (Fig. 4). Alcohol dehydrogenase activities increasingly contributed to cellular protein up to the end of the exponential phase of growth, after which the specific activities tended to decline. Greatest activity was exhibited towards octan-1-ol and least activity towards L-octan-2-ol. The ratio of activities towards octan-1-ol and D-octan-2-ol was again constant throughout growth in a particular culture medium but there was some difference between the ratio obtained in the different media. In nutrient broth and in pyruvate the ratio was constant at approx. 1.7, whilst a value of 1.2 was obtained during growth on pyruvate containing 2 mM-racemic octan-2-ol. An immediate explanation for this difference is not yet apparent. Polyacrylamide-gel electrophoresis of the cell extracts showed only two bands of activity at all stages of growth. The specificities were identical with those of the bands produced by extracts of cells grown in nutrient broth to the stationary phase.

Further experiments were performed using as culture media either nutrient broth or 1% (w/v) sodium pyruvate in basal salts solution supplemented with various alcohols or alkyl sulphate esters (see Table 1). Cells were harvested at 20 h (late exponential phase) and 38 h (late stationary phase) after inoculation and specific activities of the alcohol dehydrogenases determined as before. Table 1 shows that the specific activities noted with all three assay substrates varied little, irrespective of the nature of the growth medium. The ratio of octan-1-ol to D-octan-2-ol dehydrogenase activities was again approx. 1.2. Polyacrylamide gels of the extracts showed the usual two bands of activity. It therefore seems probable that the D-alkan-2-ol and L-alkan-2-ol dehydrogenase activities are attributable to constitutive enzymes, as various alcohols and alkyl sulphate esters were unable to affect the levels of activities produced during growth. This is a similar situation to that noted for the alkylsulphohydrolases and the adenosine 5'-phosphosulphate sulphohydrolase, which are also constitutive in *C. terrigena* (Fitzgerald *et al.*, 1975; White *et al.*, 1979). The observation contrasts with that established for *Pseudomonas* C12B where growth on nutrient broth in the presence of sodium dodecyl sulphate increased the quantity of primary alcohol dehydrogenase produced (Payne & Painter, 1971).

The collective results suggest that a single enzyme is responsible for the observed dehydrogenase activity towards D-alkan-2-ols and primary alcohols. The specificity of that enzyme would therefore be analogous to that of yeast alcohol dehydrogenase (Dickinson & Dalziel, 1967). In contrast, the L-alkan-2-ol dehydrogenase appears to be a novel enzyme not previously recognized, although stereospecific dehydrogenases active toward L-1-amino-propan-2-ol (Turner, 1967) and L- or D-butane-

Fig. 4. Production of alcohol dehydrogenase activity during growth of *C. terrigena* under different conditions Growth at 30°C on (a) nutrient broth; (b) basal salts/1% (w/v) sodium pyruvate; (c) basal salts/1% (w/v) sodium pyruvate/2 mM-DL-octan-2-ol, was monitored at 650 nm (O) for (a) and 420 nm for (b) and (c). The preparation of cell extracts and assay of alcohol dehydrogenase activity towards ■, octan-1-ol; □, D-octan-2-ol; and △, L-octan-2-ol are described in the text.

Table 1. Specific activity of the alcohol dehydrogenases in extracts of *C. terrigena* harvested at the late exponential and stationary phases following growth on various media
All defined media contained basal salts and sodium pyruvate (1%, w/v). Culture absorbances were measured at 650 and 420 nm for nutrient broth and defined media respectively.

Growth medium	Growth period (h)	Absorbance of culture	Alcohol dehydrogenase activity (units/mg of protein) towards:		
			D-Octan-2-ol	Octan-1-ol	L-Octan-2-ol
Nutrient broth	20	1.66	0.183	0.263	0.078
	38	0.95	0.097	0.106	0.023
Pyruvate	20	1.44	0.189	0.284	0.063
	38	1.52	0.134	0.150	0.040
Pyruvate + 2 mM-DL-octan-2-ol	20	1.03	0.195	0.283	0.080
	38	1.11	0.190	0.228	0.038
Pyruvate + 1 mM-D-octan-2-ol	20	1.24	0.183	0.274	0.061
	38	1.29	0.200	0.235	0.039
Pyruvate + 1 mM-L-octan-2-ol	20	1.19	0.195	0.256	0.063
	38	1.26	0.166	0.199	0.037
Pyruvate + 1 mM-octan-1-ol	20	0.96	0.182	0.249	0.056
	38	1.19	0.146	0.175	0.031
Pyruvate + 1 mM-DL-butan-2-ol	20	1.06	0.143	0.165	0.038
	38	1.91	0.093	0.112	0.018
Pyruvate + 1 mM-DL-hexan-2-ol	20	0.93	0.141	0.171	0.035
	38	1.69	0.110	0.148	0.019
Pyruvate + 1 mM-DL-octan-2-ol	20	1.03	0.143	0.190	0.039
	38	1.67	0.123	0.143	0.019
Pyruvate + 1 mM-DL-decan-2-ol	20	0.61	0.065	0.086	0.050
	38	1.49	0.066	0.086	0.019
Pyruvate + 1 mM-DL-octan-2-yl sulphate	20	1.09	0.140	0.192	0.046
	38	1.72	0.208	0.247	0.042
Pyruvate + 1 mM-octyl sulphate	20	1.06	0.167	0.193	0.051
	38	1.93	0.133	0.183	0.023

2,3-diol (Taylor & Elliot, 1960) have been reported.

C. terrigena is therefore equipped with two pairs of stereospecific enzymes for initiating the degradation of racemic alkan-2-yl sulphates. Esters of the D-configuration would be hydrolysed by the CS2 alkylsulphohydrolase to give alkan-2-ols of the L-configuration. These could then serve as substrates for L-alkan-2-ol dehydrogenase. Conversely, L-alkan-2-yl sulphates would be hydrolysed by the CS1 alkylsulphohydrolase and the liberated D-alkan-2-ols could then be oxidized by D-alkan-2-ol dehydrogenase. Primary alkyl sulphates are not degraded because the organism lacks a primary alkylsulphohydrolase enzyme to initiate the process.

Purification and further characterization of the alcohol dehydrogenases should be undertaken in order to provide additional information about the specificity and general properties of the enzymes.

Growth of C. terrigena on alcohols and related compounds

The ability of the bacterium to grow on alcohols and related compounds as sole source of carbon and energy at a concentration of 0.1% (w/v) was investigated by monitoring the turbidity of cultures at 420 nm. The organism failed to grow on octan-1-ol, heptan-4-ol or racemic samples of octan-2-ol, dodecan-2-ol, tetradecan-2-ol, hexadecan-2-ol, nonan-3-ol, hexan-2-yl sulphate, decan-2-yl sulphate or tetradecan-2-yl sulphate. Limited growth to an absorbance of 0.2 was achieved on racemic samples of butan-2-ol and pentan-2-ol, whilst slow growth to an absorbance of 1.0 occurred on butan-1-ol, pentan-1-ol, racemic octan-2-yl sulphate and octanoic acid. Attempts were made to grow cells on a selection of the foregoing compounds in the presence of 0.03% (w/v) yeast extract. Growth beyond that resulting from the utilization of the yeast extract was achieved only with butan-1-ol and racemic butan-2-ol, whilst racemic octan-2-ol diminished the growth on yeast extract and octan-1-ol inhibited it completely. The inability of *C. terrigena* to utilize many alcohols (at 0.1% concentration) as a sole carbon and energy source for growth, even though the cells possess alcohol dehydrogenase activity towards them, contrasts with the situation found for *Pseudomonas* C12B. With the exception of hexan-1-ol, heptan-1-ol and octan-1-ol, the latter organism grows well on all primary alcohols with alkyl chain-lengths of up to 18 carbons under conditions similar to those employed for *C. terrigena* (Payne, 1963; Williams *et al.*, 1966). Of course, *C. terrigena* may be unable effectively to transport the alcohols to the site of enzyme action. Possibly, growth on racemic octan-2-yl sulphate may be reflecting the release of racemic octan-2-ol within the periplasmic region of the cell, the site of localization of the CS1 and CS2 alkylsulphohydrolases (Matcham *et al.*,

1977a). Alcohols liberated in that way might therefore be more accessible to the alcohol dehydrogenases. More probably, the relatively high concentrations of alcohols used may inhibit growth of the bacterium. Growth on racemic octan-2-yl sulphate might then also be reflecting the much lower concentrations of racemic octan-2-ol present at any one time, consequent on the prior need for removal of the ester sulphate group by alkylsulphohydrolase action. If this was so, there still remains the difficulty of understanding why the organism grew on racemic octan-2-yl sulphate but not on racemic samples of decan-2-yl sulphate and tetradecan-2-yl sulphate, as both esters serve as substrates for the CS1/CS2 alkylsulphohydrolase system. Toxicity of the esters towards *C. terrigena* at the relatively high concentration employed might provide an explanation but the problem has not been pursued further.

Oxidation of alcohols and related compounds by resting cell suspensions

As *C. terrigena* appeared to be unable to utilize most primary and secondary alcohols as carbon sources for growth at 0.1% concentrations, the ability of resting cell suspensions of the micro-organism to oxidize these and related compounds was examined. Late exponential phase cells were employed after growth on either 1% (w/v) sodium pyruvate alone or supplemented with 1 mM-racemic octan-2-ol, D-octan-2-ol, L-octan-2-ol, octan-1-ol, racemic octan-2-yl sulphate or a mixture of racemic samples of octan-2-ol and octan-2-yl sulphate. Irrespective of the growth medium used, the resting cells oxidized 100 μ M-pyruvate at a rate of oxygen consumption equivalent to between 6 and 14 times that of endogenous oxygen consumption (i.e. in the absence of pyruvate). Acetate, citrate and succinate, all at concentrations of 200 μ M with respect to carbon content, were oxidized at twice the endogenous rate only, whilst primary alcohols (C_2 – C_{11}) and racemic alkan-2-ols (C_4 – C_{10}) at concentrations of 1 mM with respect to carbon, were oxidized at similar rates to acetate. Similar results were obtained for racemic and resolved isomers of octan-2-yl sulphate, octan-2-one and octanoate at the same carbon concentration. Decrease of the concentration of racemic octan-2-yl sulphate and octan-2-one to 200 μ M with respect to carbon and that of racemic butan-2-ol to 100 μ M, did not affect the rates of oxidation. It appears that resting cells of *C. terrigena* cannot rapidly oxidize any of the potential substrates (or substrate precursors) of the alcohol dehydrogenases, nor their products, irrespective of whether alcohols were initially present in the growth medium or not.

It has already been pointed out (Matcham *et al.*, 1977b) that, in contrast to *Pseudomonas* C12B, *C.*

terrigena could have a limited capacity only to degrade alkylsulphate detergents. Under the most favourable circumstances, degradation would be restricted to detergents containing alkan-2-sulphates. On the other hand, the ability of *Pseudomonas* C12B to produce two primary alkylsulphohydrolases and three secondary alkylsulphohydrolases of differing specificity but which collectively could degrade most secondary alkylsulphates, provides the organism with a much greater capacity for degrading detergents containing alkylsulphate esters. Apart from the initial observations of Williams *et al.* (1966) and Payne & Painter (1971) which established the presence of NAD-dependent dehydrogenase activity towards primary and some secondary alcohols, no detailed studies have been made on the alcohol dehydrogenases of *Pseudomonas* C12B. In view of its capacity to produce five alkylsulphohydrolases it will now be of considerable interest to investigate the specificity and complexity of alcohol dehydrogenase activity in this bacterium.

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References

- Bartholomew, B., Dodgson, K. S., Matcham, G. W. J., Shaw, D. J. & White, G. F. (1977) *Biochem. J.* **165**, 575–580
- Dickinson, F. M. & Dalziel, K. (1967) *Nature (London)* **214**, 31–33
- Dodgson, K. S., Fitzgerald, J. W. & Payne, W. J. (1974) *Biochem. J.* **138**, 53–62
- Fitzgerald, J. W. & Payne, W. J. (1972) *Microbios* **5**, 87–100
- Fitzgerald, J. W., Dodgson, K. S. & Matcham, G. W. J. (1975) *Biochem. J.* **149**, 477–480
- Grell, E. H., Jacobson, K. B. & Murphy, J. B. (1965) *Science* **149**, 80–82
- Lijmbach, G. W. M. & Brinkhuis, E. (1973) *Antonie van Leeuwenhoek* **39**, 415–423
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Matcham, G. W. J. & Dodgson, K. S. (1977) *Biochem. J.* **167**, 717–722
- Matcham, G. W. J., Dodgson, K. S. & Fitzgerald, J. W. (1977a) *Biochem. J.* **167**, 723–729
- Matcham, G. W. J., Bartholomew, B., Dodgson, K. S., Fitzgerald, J. W. & Payne, W. J. (1977b) *FEMS Microbiol. Lett.* **1**, 197–200
- Payne, W. J. (1963) *Biotechnol. Bioeng.* **5**, 355–365
- Payne, W. J. & Painter, B. G. (1971) *Microbios* **3**, 199–206
- Taylor, M. B. & Elliott, J. (1960) *Biochim. Biophys. Acta* **39**, 448–457
- Turner, J. M. (1967) *Biochem. J.* **104**, 112–121
- White, G. F., Rowlands, M. G., Dodgson, K. S. & Payne, W. J. (1979) *FEMS Microbiol. Lett.* **5**, 267–271
- Williams, J. & Payne, W. J. (1964) *Appl. Microbiol.* **12**, 360–362
- Williams, J. P., Mayberry, W. R. & Payne, W. J. (1966) *Appl. Microbiol.* **14**, 156–160