Arch Microbiol (1988) 150:138–144

Archives of
Microbiology

Isolation of transposon Tn*5* insertion mutants of *Rhodobacter capsulatus* unable to reduce trimethylamine-N-oxide and dimethylsulphoxide

D. J. Kelly[1,*], D. J. Richardson[1], S. J. Ferguson[2], and J. B. Jackson[1]

[1] Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham, B15 2TT, UK
[2] Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, UK

Abstract. 1) *Rhodobacter capsulatus* (formerly *Rhodopseudomonas capsulata*) strain 37b4 was subjected to transposon Tn5 mutagenesis. 2) Kanamycin-resistant transconjugants were screened for their inability to reduce trimethylamine-N-oxide (TMAO) as judged by the lack of alkali production during anaerobic growth on plates containing glucose as carbon source and cresol red as pH indicator. 3) Of 6 mutants examined, all were found to have considerably decreased levels of methylviologen-dependent TMAO reductase activity and dimethylsulphoxide (DMSO) reductase activity. 4) Periplasmic fractions of one of these mutants (DK9) and of the parent strain were subjected to sodium dodecylsulphate polyacrylamide gel electrophoresis. The gels were stained for TMAO-reductase and DMSO-reductase. With the wild-type strain, only a single polypeptide band, $M_r = 46{,}000$, stained for TMAO and DMSO reductase activity. In mutant DK9 this band was not detectable. 5) In contrast to the parent strain, harvested washed cells of mutant DK9 were unable to generate a cytoplasmic membrane potential in the presence of TMAO or DMSO under dark anaerobic conditions. 6) In contrast to the parent strain, DK9 was unable to grow in dark anaerobic culture with fructose as the carbon source and TMAO as oxidant.

Key words: Photosynthetic bacteria (*Rhodobacter capsulatus*) – Transposon mutagenesis – TMAO reduction – DMSO reduction – Anaerobic respiration – Electron transport

Photosynthetic bacteria of the family Rhodospirillaceae are well known for their metabolic versatility (Pfennig 1977). This has been underlined in recent years by the discovery that certain species, notably *Rhodobacter* (Rb.) *capsulatus* (formerly *Rhodopseudomonas capsulata*) are able to grow under anaerobic dark conditions when provided with suitable carbon sources, especially sugars, and "accessory oxidants" including trimethylamine-N-oxide (TMAO) or dimethylsulphoxide (DMSO) (Yen and Marrs 1977; Madigan and Gest 1978). During growth, TMAO and DMSO are reduced to trimethylamine and dimethylsulphide respectively. It was initially concluded that these reductions were not linked to energy conservation (Cox et al. 1980), but rather acted as electron sinks in order to maintain the correct redox balance during the fermentation of sugars under dark, anaerobic conditions (Cox et al. 1980).

In an extensive study of many strains, however, Schulz and Weaver (1982) found that *Rb. capsulatus* was capable of anaerobic dark growth with non-fermentable substrates such as malate or succinate provided that either TMAO or DMSO were also present as terminal electron acceptors. Moreover, McEwan et al. (1983) demonstrated that the addition of TMAO or DMSO to cell suspensions of *Rb. capsulatus*, which had been grown under anaerobic, dark conditions, led to the generation of a rotenone- and uncoupler-sensitive membrane potential ($\Delta\Psi$). These findings clearly indicate the operation of a proton-translocating electron transport chain during reduction of TMAO or DMSO.

More recently, it has been shown that cells grown phototrophically with malate and TMAO or DMSO in the medium also exhibit reductase activity for these oxides and that the enzyme system responsible for this activity is located in the periplasm of *Rb. capsulatus* (McEwan et al. 1985b). In addition, activity staining of polyacrylamide gels run under both denaturing and non-denaturing conditions suggest that the same enzyme might be responsible for reduction of both TMAO and DMSO (McEwan et al. 1985b, 1987). Although the role of these electron acceptors under phototrophic conditions is unclear, one function (Ferguson et al. 1987; McEwan et al. 1985c) could be to allow the cells to achieve an optimal redox poise of the cyclic, photosynthetic electron transport chain. This may be especially important during growth on reduced carbon sources (Richardson et al. 1988). The information about the roles of TMAO and DMSO reduction now needs to be complemented by more data on the molecular nature of both the reductase and the proteins that are involved with electron transport to TMAO and DMSO reductase. As part of such a study, we have used the technique of transposon mutagenesis with Tn*5* (Kaufman et al. 1984), combined with the development of a suitable screening method, to isolate mutants of *Rb. capsulatus* that are deficient in the reduction of TMAO

* *Present address:* Department of Microbiology, University of Sheffield, Western Bank, Sheffield, S10 2TN, UK

Offprint requests to: J. B. Jackson

Abbreviations: TMAO, trimethylamine-N-oxide; DMSO, dimethylsulphoxide; PMS, phenazine methosulphate; $\Delta\psi$, cytoplasmic membrane potential

Table 1. Strains and plasmids used in this study

Strain/plasmid	Characteristics	Source of reference
E. coli		
FE62 (RP4)	km^r, Ap^r, Tc^r	Datta et al. 1971
S17-1 (pSUP2021; pBr325:: Tn*5*::*mob*)	km^r, Ap^r, Cm^r	Simon et al. 1983
Rb. capsulatus		
37b4	T/DOR^+, Str^r	H. Hudig
AD2	T/DOR^+, $DNAR^+$, $NOSR^+$	J. H. Klemme
N22	T/DOR^+, $NOSR^+$	O.T.G. Jones
St. Louis	T/DOR^+, $NOSR^+$	H. Hudig

km, kanamycin; *Ap*, ampicillin; *Tc*, tetracycline; *Cm*, chloramphenicol; *Str*, streptomycin; T/DOR^+, reduction of TMAO/DMSO; $DNAR^+$, dissimilatory nitrate reduction; $NOSR^+$, reduction of nitrous oxide

and DMSO. Some characteristics of these mutants are described.

Methods

Bacterial strains and growth conditions. Table 1 gives details of the strains used in this study. *Escherichia coli* was grown aerobically in 50 ml volumes of nutrient broth (Oxoid) contained in 250 ml flasks on a rotary shaker (275 r.p.m.) at 30° C.

Resistance to antibiotics was checked by testing aerobic growth at 37° C on nutrient agar plates containing either kanamycin, ampicillin or chloramphenicol at 25 μg/ml final concentration, or tetracycline at 10 μg/ml final concentration. *Rb. capsulatus* was grown photosynthetically in RCV medium (Weaver et al. 1975) as described previously (McEwan et al. 1983). Where required, additions of DMSO or TMAO were from filter-sterilised stock solutions to give 30 mM final concentration. For chemoheterotrophic growth, 50–100 ml volumes of RCV contained in 250 ml flasks were inoculated (1% v/v) with *Rb. capsulatus* and incubated on a rotary shaker at 250 r.p.m. and 30° C. RCV agar plates were incubated aerobically at 30° C and, where required, streptomycin or kanamycin was added to 25 μg/ml final concentration.

Conjugations. Tn*5* mutagenesis of *Rb. capsulatus* was carried out essentially as described by Kaufman et al. (1984). Overnight cultures of *E. coli* and chemoheterotrophically grown *Rb. capsulatus* were harvested aseptically by centrifugation (8,000 × *g*, 20 min, 30° C) and each was resuspended in 1 ml of RCV medium. The two suspensions were mixed to give an approximate ratio of donor:recipient of 1:1 and 0.4–0.6 ml aliquots were applied to membrane filters (Millipore 0.45 μm) on the surface of RCV plates containing 0.1% (w/v) yeast extract (RCVYE). Conjugation was for 5 h at 30° C. The cells were then washed off the filters by resuspension and vigorous vortex mixing in 1 ml of RCV medium. Serial dilutions were made in RCV medium and 10 μl aliquots plated out on RCV plates alone (for viable counts) or on RCV containing 25 μg/ml kanamycin. Plates were incubated for 4–5 days at 30° C under aerobic conditions. As a control, *Rb. capsulatus* was also incubated alone on membrane filters under the above conditions to check for spontaneous resistance to kanamycin.

Screening procedure. Kanamycin resistant transconjugants were stabbed into a modified RCV medium (TMAO/CR) containing glucose (20 mM) as carbon source, 0.05–0.10% (wv) cresol red as a pH indicator, 30 mM TMAO and 25 μg/ml kanamycin. The medium was solidified with 1.5% (w/v) Difco bacto-agar. Filter sterilised solutions of TMAO and kanamycin were added after autoclaving the medium. After 2–3 days aerobic incubation in the dark at 30° C, blocks of agar containing stabs which were not surrounded by red coloured zones were transferred to 5 ml RCV medium contained in filled Bijoux bottles and incubated phototrophically at 30° C. After growth had occurred, cells were streaked out onto RCV plates containing 25 μg/ml kanamycin.

Measurement of TMAO/DMSO oxidoreductase activities. Cells from phototrophically grown cultures were harvested by centrifugation, (10,000 × *g*, 20 min, 4° C) washed once and resuspended with RCV containing no nitrogen source (RCV-N). The rate of reduction of TMAO, DMSO or substrate analogues was determined from the rate of oxidation of dithionite-reduced methyl viologen in a dual-wavelength spectrophotometer (McEwan et al. 1985b). The wavelength pair used was 600–530 nm with an extinction coefficient of 6.50 mM^{-1} cm^{-1}.

Polyacrylamide gel electrophoresis. Periplasmic fractions were prepared from phototrophically grown cells as described by McEwan et al. (1985b), dialysed against 10 mM Tris HCl pH 7.0 at 4° C overnight, and then concentrated 20-fold with Aquacide (Calbiochem.). For activity staining, samples were denatured at room temperature in Laemmli sample buffer (Laemmli 1970) and electrophoresed on a 12.5% gel (acrylamide: bis-acrylamide ratio 30:0.8) for 16 h at 10° C. Gels were stained for reductase activity according to Shimokawa and Ishimoto (1979).

Measurement of membrane potentials. The development of membrane potential in whole cells in response to either illumination or the addition of auxiliary oxidants was monitored using the electrochromic response of the endogeneous carotenoids as described previously (McEwan et al. 1983, 1984). The wavelength pair was 528–511 nm.

Analytical procedures. Bacteriochlorophyll was determined after extraction of whole cells into acetone:methanol (7:2 v/v) using an extinction coefficient of 75 mM^{-1} cm^{-1} at 772 nm (Clayton 1963). Protein was determined by the Lowry method (Lowry et al. 1951).

Results

Transfer of plasmids to strains of Rhodobacter capsulatus

Previous work from this laboratory on anaerobic respiration, including reduction of DMSO and TMAO, has been done using N22 and AD2 strains of *Rb. capsulatus* (McEwan et al. 1983, 1984, 1985a, b, c, 1987). However, the capacity of different strains to accept plasmids *via* conjugation has not been studied extensively. Therefore, we initially tested

Table 2. Transfer of plasmids by conjugation to strains of *Rb. capsulatus*

Strain	km^r frequency (per recipient) after conjugation of plasmid		
	RP4	pSUP2021	None
37b4	3.3×10^{-2} (4)	3.5×10^{-6} (4)	$<10^{-10}$ (3)
St. Louis	2.0×10^{-4} (3)	5.0×10^{-8} (2)	$<10^{-10}$ (3)
N22	6.6×10^{-5} (1)	5×10^{-9} (1)	$<5\times10^{-9}$ (1)
AD2	2.0×10^{-6} (3)	10^{-9} (5)	$<5\times10^{-9}$ (2)

Frequencies represent the average value obtained from the number of determinations (separate matings) given in parentheses

the suitability of selected strains of *Rb. capsulatus* for transposon mutagenesis by determining the frequency of kanamycin resistant transconjugants after transfer of the plasmid pSUP2021 (Table 2). For comparison, we also determined such frequencies for the broad-host range plasmid RP4, which is known to be stably maintained in this species (Yu et al. 1981).

Conjugal transfer of pSUP2021 resulted in a low kanamycin resistance frequency in most strains, only one or two orders of magnitude above the background, spontaneous value. This can be attributed to low mating frequencies or DNA restriction, because the corresponding k_m resistance frequencies obtained after transfer of RP4 also followed the same pattern (Table 2). However, *Rb. capsulatus* strain 37b4 proved a good recipient for Tn*5*, with a k_m resistance frequency approaching 10^{-6} with pSUP2021. This strain has been well characterised (see Drews and Oelze 1981) and transfer of pSUP2021 into it has been demonstrated in a previous study to result in both loss of the plasmid and the generation of single chromosomal Tn*5* insertions (Kaufman et al. 1984). It was therefore selected for mutagenesis, aftere ensuring that it possessed TMAO and DMSO reductase activities.

Development of a screening method for mutants defective in TMAO reduction

It was observed that when *Rb. capsulatus* was grown with glucose as carbon source an alkaline pH change only occurred in the presence of TMAO. TMAO reduction thus appeared to neutralise the acid end products normally resulting from glucose metabolism. Such an increase in pH was detected as a red zone around individual colonies growing on solid media (pH 7.0) containing TMAO and the pH-indicator cresol red (pK_a 7.8). Failure to produce red zones could thus be used to detect colonies that lacked TMAO reductase. For convenience, plates were incubated aerobically and under these conditions red zones developed most extensively when cells were stabbed into the agar to allow local anaerobiosis to develop during growth.

From six independent filter matings, a total of 2,950 km^r transconjugants were randomly picked from selection plates and screened for TMAO reduction using the aforementioned method. Putative mutants exhibiting the desired phenotype were restreaked 5 times from single colonies on RCV plates containing kanamycin and then tested again for trimethylamine production on TMAO/cresol red plates. Of 15 putative mutants, six still exhibited both km^r and lack of trimethylamine production after re-purification, giving an overall mutant frequency of 0.20%.

Table 3. Methylviologen-dependent reductase activities in whole cells of *Rb. capsulatus* wild type 37b4 and in mutants

Strain	Enzyme activity (μmol mv oxidised min^{-1} μmol^{-1} BChl)	
	Electron acceptor	
	TMAO	DMSO
37b4 (wild-type) (DMSO grown)	14.0	5.8
37b4 (TMAO grown)	30.5	12.9
DK9 (Tn*5*) (TMAO grown)	1.46	ND
DK10 (Tn*5*) (TMAO grown)	0.61	ND
DK14 (Tn*5*) (TMAO grown)	1.40	ND
DR6 (Tn*5*) (TMAO grown)	1.56	ND
DR7 (Tn*5*) (TMAO grown)	0.97	ND
DR 8 (Tn*5*) (TMAO grown)	1.00	ND

Cells (2 or 20 μm BChl) were added to 2.5 ml RCV-N containing 200 μM methylviologen. The dye was reduced by titrating in small aliquots (5–15 μl) of a sodium dithionite solution to give a stable absorbance reading. The reaction was started by addition of electron acceptor to 2 mM and the decrease in absorbance at 600–530 nm was recorded. The reaction mixture was contained in a stirred cuvette with facilities for gassing with argon (< 3 ppm O_2) to maintain anaerobiosis. All cells were harvested from phototrophically grown cultures (RCV + malate) plus the electron acceptor shown. ND = not detectable

Terminal oxidoreductase activities of mutants defective in TMAO reduction

Table 3 shows the reductase activities obtained in whole cells of wild type and mutant derivatives after phototrophic growth with malate as carbon source and in the presence of either DMSO or TMAO. The highest activities were observed with wild type cells reducing TMAO or its analogue pyridine-N-oxide which was reduced at approximately 40% of the rate for TMAO. The activities with DMSO or the analogue methionine sulphoxide were similar to each other but much lower than with TMAO. Essentially the same pattern of activities was seen when the cells were grown on RCV + DMSO.

Each of the six mutants examined showed only traces of TMAO reductase activity (2–5% of the wild type) and no activity with DMSO or, where studied, any analogue. These data suggest that the mutants are unable to reduce TMAO because of a deficiency in the production or function of the terminal reductase to which reduced viologens are thought to be electron donors. As the phenotype of the six mutants appeared similar, further studies were largely carred out using just one mutant, DK9.

Activity staining of periplasmic fractions on denaturing polyacrylamide gels

To investigate further the lesion in mutant DK9, periplasmic fractions were prepared from wild type and mutant cells

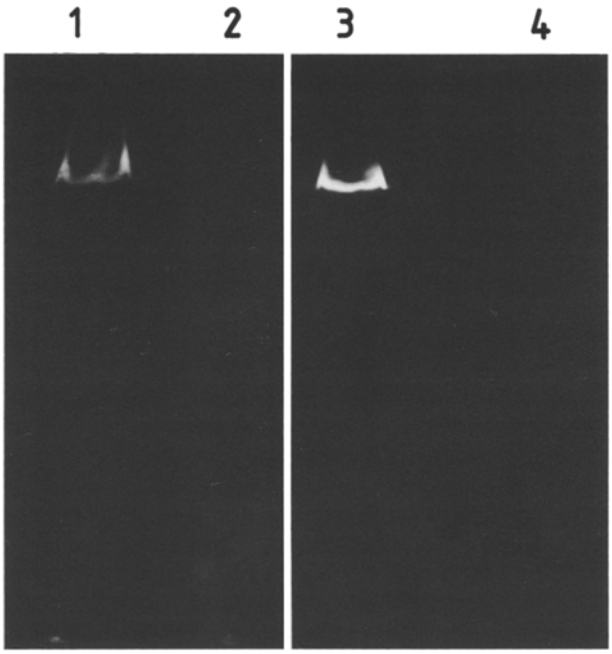

Fig. 1. Sodium dodecylsulphate polyacrylamide gel electrophoresis of periplasmic fractions of *Rb. capsulatus* 37b4 and DK9: staining for TMAO- and DMSO-reductase. See Methods. 300 µg protein was applied per track. *Track 1*, periplasm from 37b4 stained for TMAO reductase; *track 2*, periplasm from DK9 stained for TMAO reductase; *track 3*, periplasm form 37b4 stained for DMSO reductase; *track 4*, periplasm form DK9 stained for DMSO reductase

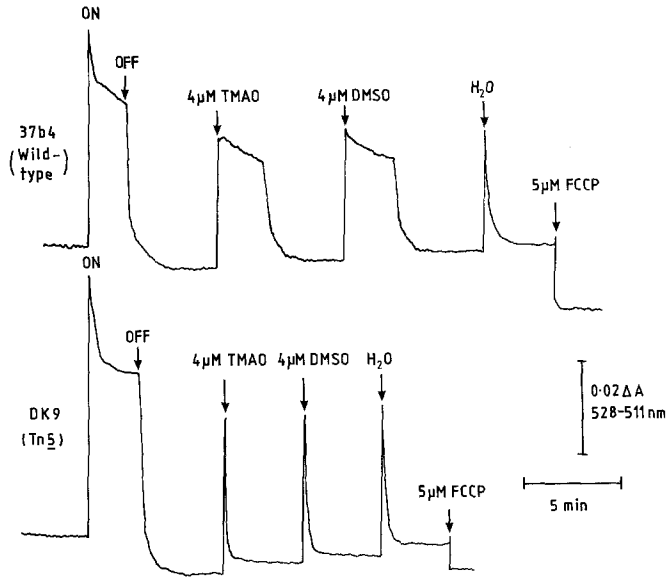

Fig. 2. Generation of membrane potential in intact cells of *Rb. capsulatus* 37b4 and DK9. Harvested and washed cells were resuspended in fresh RCV medium to give 20 µM bacteriochlorophyll and incubated in the dark under argon in the spectrophotometer cuvette for approximately 30 min. Photosynthetic illumination was supplied as indicated and additions of TMAO and DMSO were made as concentrated aqueous solutions to give the final concentration shown. Carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) was added at the end of the experiment to deenergise completely the cell suspensions

after growth under phototrophic conditions with malate and TMAO. Samples of the periplasmic fractions from the wild type had methyl viologen-dependent, TMAO- and DMSO-reductase activities of 1,248 and 230 nmol · min^{-1} · mg^{-1} protein respectively. For DK9, activities were 80 and 0 respectively. Further samples of the periplasmic fractions were subjected to polyacrylamide gel electrophoresis (Fig. 1). Only one polypeptide band, of M_r 46,000, from the periplasm of the wild type stained for TMAO reductase. No polypeptide band stained for activity in the periplasmic fraction from mutant DK9. Importantly, the band from periplasmic fraction from the wild type that stained for DMSO reductase activity had an identical mobility to the band staining for TMAO reductase, whereas no polypeptide from the DK9 mutant stained for DMSO reductase activity (Fig. 1). In confirmatin of earlier work (McEwan et al. 1987) the apparent molecular weight of this band was approx. 46,000.

Reductase mutants are unable to generate a membrane potential upon addition of either TMAO or DMSO

Figure 2 shows an experiment in which harvested and washed cells of *Rb. capsulatus* were incubated under anaerobic conditions and monitored for the development of membrane potential by the electrochromic response of the endogeneous carotenoids. Illumination with actinic light caused an absorbance change in both wild type and mutant cells which signified the generation of a cytoplasmic membrane potential. However, under dark conditions, the addition of TMAO or DMSO resulted in the formation of a membrane potential only in wild type cells. Addition of either TMAO and DMSO to DK9 cells incubated under the same conditions, led only to transient absorption changes which were also observed after the addition of a similar quantity of distilled water. Such changes represent addition artefacts due to birefringence changes or traces of dissolved oxygen. It may be noted that because of the non-linear ionic conductance of the bacterial cytoplasmic membrane (Clark et al. 1983) the electrochromic absorbance change is an extremely sensitive indicator for the existence of coupled electron transport. The complete absence of any TMAO-dependent membrane potential formation in strain DK9 is, therefore, firm evidence that the coupled pathway to TMAO in vivo is inoperative. This might indicate that the residual TMAO-reductase activity in the methyl viologen assay in this strain (Table 3) is artefactual and is not a result of a low level of expression of the enzyme. Similar observations of a low level of methylviologen-dependent TMAO-reductase (Table 3) and an absence of TMAO-induced electrochromic absorbance change (data not shown) were also found for strains DR6, DR7 and DR8.

Restoration of disturbed redox poise by TMAO in Rb. capsulatus mutant DK9

Recently it was shown that the addition of low concentrations of the redox mediator PMS to anaerobic suspensions of intact cells of *Rb. capsulatus* led to inhibition of the cyclic photosynthetic electron transport chain (McEwan et al. 1985b). This was probably the result of over-reduction of the electron transport carriers as a result of a short-circuit in the homeostatic mechanism which must normally prevent redox equilibration with the low potential nucleotide pools in the cell cytoplasm (Cotton and Jackson 1982). The effect

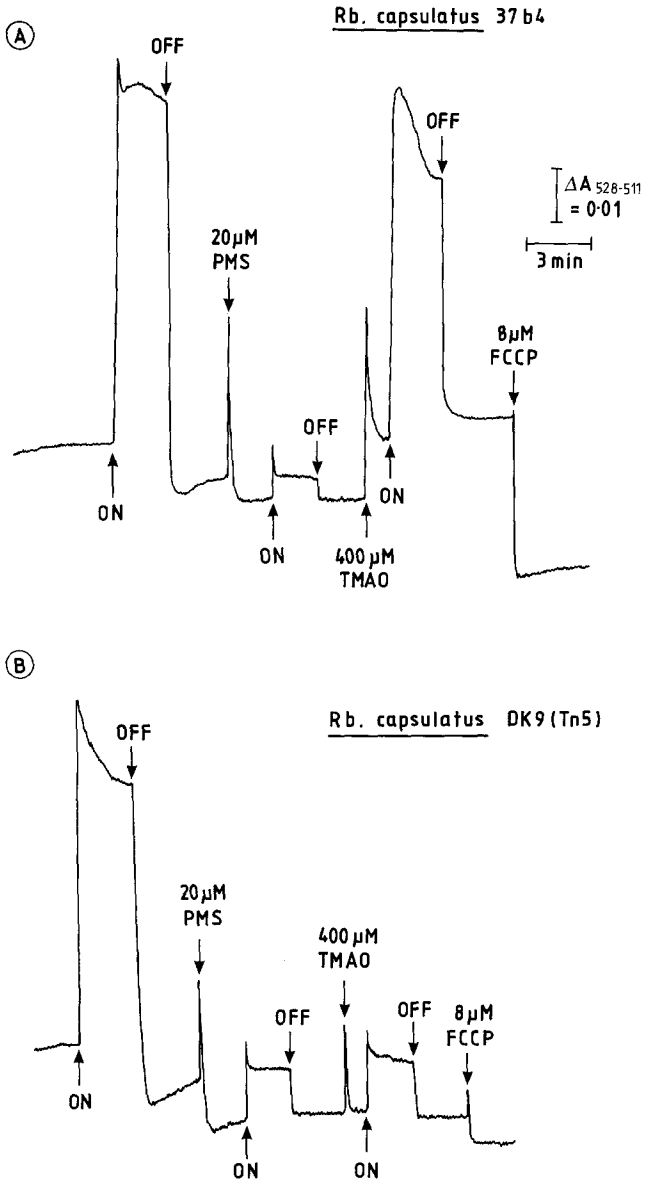

Fig. 3A, B. Redox poising in *Rb. capsulatus* strains 37b4 and DK9 after perturbation by phenazine methosulphate. Conditions as in Fig. 2. Phenazine methosulphate, TMAO and FCCP were added where indicated

is demonstrated in the parent strain, 37b4, in Fig. 3 in which electrochromic absorbance changes are used primarily as an index of the activity of the photosynthetic electron transport chain: the addition of PMS had a pronounced inhibitory effect on the development of membrane potential during a brief period of illumination and the inhibition was largely counteracted by a subsequent addition of TMAO. In mutant DK9 the addition of TMAO did not lead to restoration of the light-induced $\Delta\Psi$ after inhibition by PMS.

Comparison of growth of wild type and mutant strains with TMAO as an electron acceptor. Rb. capsulatus can grow anaerobically in the dark with fructose only in the presence of an auxiliary oxidant such as TMAO (Madigan and Gest 1978). This was confirmed with *Rb. capsulatus* strain 37b4 which showed a doubling time in exponential phase of approx. 10 h. The Tn*5* mutant, DK9, was unable to grow under these conditions either in the presence or absence of TMAO (data not shown).

Discussion

Screening for Tn5 insertion mutants of Rb. capsulatus deficient in the reduction of TMAO. The screening procedure used in this study is a relatively simple one, based on the alkaline pH change accompanying the production of trimethylamine from TMAO. An analogous method has previously been used with *E. coli* and *Salmonella typhimurium* (Davidson et al. 1979; Kwan and Barrett 1983) by screening for mutants which produce an acid reaction on McConkey-glucose-TMAO plates, rather than monitoring the alkaline pH change due to TMA production directly.

In principle at least four classes of *Rb. capsulatus* mutants might be isolated by this screening procedure. These are (i) mutants which lack the structural proteins of the terminal TMAO-reductase (ii) those deficient in functional electron donor(s) proteins to the terminal enzyme (iii) mutants unable to synthesise or process particular prosthetic groups/ cofactor(s) associated with the terminal enzyme or electron donor(s) and (iv) mutants affected in the regulation of synthesis of the latter proteins.

The mutants obtained in this study all appeared to exhibit the same phenotype – a deficiency in the production or function of the terminal TMAO oxidoreductase. It is not yet known if these are regulatory mutants or whether they fall into class (i) or (iii), as defined above. The very low TMAO-dependent methylviologen oxidation observed in whole cells and the absence of activity staining of the appropriate polypeptide on polyacrylamide gels, is evidence against the possibility of an electron donor mutation, because Tn*5* insertions should be at a single site in this microbe (Kaufman et al. 1984). However, Tn*5* insertion may cause polar effects on downstream genes (Kleckner et al. 1977) and so a pleiotropic phenotype cannot be entirely ruled out.

It is important to note that the type of screening procedure used will also have an affect on the types of mutants obtained. Thus, if the electron donor(s) to TMAO reductase were common to the respiratory electron transport chain, for example, then an aerobic screening procedure would be unsuitable, because such mutants would be non-viable under these conditions. Alternatively, if two or more alternative electron pathways to the TMAO reductase existed, then a single mutation arising from Tn*5* insertion may not be identifiable using the screening method employed here. Similar considerations were advanced by Zumft et al. (1985) to account for the absence of electron donor mutations amongst Tn*5* induced mutants of *Pseudomonas perfectomarinus* unable to reduce nitrous oxide.

Evidence that TMAO and DMSO reduction is catalysed by a single enzyme

In previous papers (McEwan et al. 1985b, 1987) it has been concluded that TMAO and DMSO are reduced by a common enzyme. This was based on evidence from activity staining of both non-denaturing and denaturing gels.

Two pieces of evidence obtained in this study strongly support the suggestion that TMAO and DMSO are substrates for the same enzyme; (i) mutants selected specifically for the inability to reduce TMAO were also unable to reduce

DMSO, or the respective analogues, pyridine-N-oxide and methionine sulphoxide, and (ii) activity staining of denaturing polyacrylamide gels revealed that one polypeptide in the wild type of apparent M_r 46,000, which reduced both TMAO and DMSO, was not active in the mutant. This polypeptide is therefore presumably the catalytic subunit of the TMAO/DMSO oxidoreductase. As yet, little is known about the properties of this enzyme from *Rb. capsulatus*, since it has not been purified to homogeneity. However, it appears to be a molybdoenzyme (McEwan et al. 1987) in common with the enzyme from *E. coli* (Yamamoto et al. 1986). There is no evidence from the present work to suggest that more than one TMAO reductase exists in *Rb. capsulatus*, while *E. coli* appears to possess several different forms of TMAO reductase (Shimokawa and Ishimoto 1979). A single TMAO/DMSO reductase has also been reported in *Proteus vulgaris* (Styrvold and Strom 1984).

Further applications of the screening procedure

Previous studies have shown that some strains of *Rb. capsulatus* are capable of reducing not only TMAO/DMSO but also nitrate (McEwan et al. 1982) and nitrous oxide (McEwan et al. 1985a). The screening procedure introduced here would be useful in obtaining mutants which are simultaneously deficient in the reduction of two, or more, of the above oxidants. Such pleiotropic mutants, if obtainable, might be useful in the identification of electron transport components which are common to the various pathways. Unfortunately, 37b4 does not contain detectable levels of either nitrate or nitrous oxide reductase activities (unpublished observations) and therefore it is not suitable for selection of pleiotropic mutants in such pathways. However, the continued application of Tn*5* mutagenesis to *Rb. capsulatus* should allow the isolation of a variety of useful mutants which will yield important information on the organisation, regulation and expression of auxiliary electron transport pathways in this microbe.

Acknowledgements. We thank the U.K. Science and Engineering Research Council for support of this work through grants to SJF and JBJ, and thereby a fellowship to DJK and a studentship to DJR. We are grateful to H. Hüdig and R. Simon for providing strains.

References

Clark AJ, Cotton NPJ, Jackson JB (1983) The influence of the ionic conductance on the relation between electron transport and protonmotive force in intact cells of *Rhodopseudomonas capsulata*. Eur J Biochem 130:575–580

Clayton RK (1963) Towards the isolation of a photosynthetic reaction centre in *Rhodopseudomonas capsulata*. Biochim Biophys Acta 73:312–323

Cotton NPJ, Jackson JB (1982) Kinetics of carotenoid absorption changes in intact cells of photosynthetic bacteria. Biochim Biophys Acta 679:138–145

Cox JC, Madigan MT, Faringer JF, Gest H (1980) Redox mechanisms in "oxidant-dependent" hexose fermentation by *Rhodopseudomonas capsulata*. Arch Biochem Biophys 204:10–17

Datta N, Hedges RW, Shaw EJ, Sykes RB, Richmond MH (1971) Properties of an R factor from *Pseudomonas aeruginosa*. J Bacteriol 108:1244–1249

Davidson AE, Fukimoto HE, Jackson CE, Barrett EL, Chang GW (1979) Mutants of *Salmonella typhimurium* defective in the reduction of trimethylamine oxide. FEMS Microbiol Lett 6: 417–420

Drews G, Oelze J (1981) Organisation and differentiation of membranes of phototrophic bacteria. Adv Microbial Physiol 22:1–92

Ferguson SJ, Jackson JB, McEwan AG (1987) Anaerobic respiration in the Rhodospirillacae: characterisation of pathways and evaluation of roles in redox balancing during photosynthesis. FEMS Microbiol Rev 46:117–143

Kaufmann N, Hudig H, Drews G (1984) Transposon Tn*5* mutagenesis of genes for the photosynthetic apparatus in *Rhodopseudomonas capsulata*. Mol Gen Genet 198:153–158

Kleckner N, Roth J, Botstein D (1977) Genetic engineering *in vivo* using translocatable drug resistance elements. J Mol Biol 116:125–159

Kwan HS, Barrett EL (1983) Roles for menaquinone and the two trimethylamine oxide (TMAO) reductases in TMAO respiration in *Salmonella typhimurium*: Mad (Ap^r*lac*) insertion mutations in *men* and *tor*. J Bacteriol 115:1147–1155

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the lead of bacteriophage T4. Nature (Lond.) 227:680–685

Lowry OH, Rosebrough HJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Madigan MR, Gest H (1978) Growth of photosynthetic bacteria anaerobically in darkness, supported by "oxidant-dependent" sugar fermentation. Arch Microbiol 117:119–122

McEwan AG, George CL, Ferguson SJ, Jackson JG (1982) A nitrate reductase activiy in *Rhodopseudomonas capsulata* linked to electron transfer and generation of a membrane potential. FEBS Lett 150:277–280

McEwan AG, Ferguson SJ, Jackson JB (1983) Electron flow to dimethylsulphoxide or trimethylamine-N-oxide generates a membrane potential in *Rhodopseudomonas capsulata*. Arch Micrbiol 136:300–305

McEwan AG, Jackson JB, Ferguson SJ (1984) Rationalisation of properties of nitrate reductases in *Rhodopseudomonas capsulata*. Arch Microbiol 137:344–349

McEwan AG, Greenfield AJ, Wetzstein HG, Jackson JB, Ferguson SJ (1985a) Nitrous oxide reduction by members of the family *Rhodospirillaceae* and the nitrous oxide reductase of *Rhodopseudomonas capsulata*. J Bacteriol 164:823–830

McEwan AG, Wetzstein HG, Ferguson SJ, Jackson JB (1985b) Periplasmic location of the terminal reductase in trimethylamine-N-oxide and dimethylsulphoxide respiration in the photosynthetic bacterium *Rhodopseudomonas capsulata*. Biochim Biophys Acta 806:410–417

McEwan AG, Cotton NPJ, Ferguson SJ, Jackson JB (1985c) The role of auxiliary oxidants in the maintencance of a balanced redox poise for photosynthesis in bacteria. Biochim Biophys Acta 810:140–147

McEwan AG, Wetzstein HG, Meyer O, Jackson JB, Ferguson SJ (1987) The periplasmic nitrate reductase of *Rhodobacter capsulatus*; purification, characterisation and distinction from a single reductase for trimethylamine-N-oxide, dimethylsulphoxide and chlorate. Arch Microbiol 147:340–345

Pfennig N (1977) Phototrophic green and purple bacteria: a comparative systematic survey. Microbiology 31:275–290

Richardson DJ, Kelly DJ, Jackson JB, Ferguson SJ, Alef K (1986) Inhibitory effects of myxothiazol and 2-n-heptyl-4-hydroxquinoline-N-oxide on the auxiliary electron transport pathways of *Rhodobacter capsulatus*. Arch Microbiol 146:159–165

Richardson DJ, King GF, Kelly DJ, McEwan AG, Ferguson SJ, Jackson JB (1988) The role of auxiliary oxidants in maintaining redox balance during phototrophic growth of *Rhodobacter capsulatus* on reduced carbon substrates. Arch Microbiol 150:131–137

Schulz JE, Weaver PF (1982) Fermentation and anaerobic respiration by *Rhodospirillum rubrum* and *Rhodopseudomonas capsulata*. J Bacteriol 149:181–190

Shimokawa B, Ishimoto I (1979) Purification and some properties of inducible tertiary amine-N-oxide reductase from *E. coli*. J Biochem 86:1709–1717

Simon R, Priefer V, Peuhler A (1983) Vector plasmids for *in vivo* and *in vitro*, manipulations of Gram-negative bacteria. In: Peuhler A (ed) Molecular biology of bacteria plant interaction. Springer, Berlin Heidelberg New York p 99

Styrvold OB, Strom AR (1984) Dimethyl sulphoxide and trimethylamine-N-oxide respiration of *Proteus vulgaris*. Arch Microbiol 140:74–78

Weaver PF, Wall JD, Gest H (1975) Characterisation of *Rhodopseudomonas capsulata*. Arch Microbiol 105:207–216

Yamamoto J, Okibo N, Ishimoto M (1986) Further characterisation of trimethylamine-N-oxide reductase from *Escherichia coli*, a molybdoprotein. J Biochem (Tokyo) 99:1773–1779

Yen H-C, Marrs BL (1977) Growth of *Rhodopseudomonas capsulata* under anaerobic dark conditions with dimethylsulphoxide. Arch Biochem Biophys 181:411–418

Yu PL, Cullum J, Drews G (1981) Conjugational transfer systems of *Rhodopseudomonas capsulata* mediated by R plasmids. Arch Microbiol 128:390–393

Zumft WG, Dohler K, Korner H (1985) Isolation and characterisation of transposon Tn*5*-induced mutants of *Pseudomonas perfectomarina* defective in nitrous oxide respiration. J Bact 613:918–924

Received December 17, 1987/Accepted March 3, 1988