Linkage Relationships Between Mutants of *Methylobacterium organophilum* Impaired in their Ability to Grow on One-carbon Compounds

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Eight mutant strains of *Methylobacterium organophilum* which are capable of growing on succinate but not on methanol are described. Three of these contain single enzyme deficiencies while five have pleiotropic deficiencies. Some of the mutant strains are revertible to the wild-type phenotype by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and ethyl methanesulphonate and others are not.

Crosses between strains were performed by transformation using DNA prepared from cells which were streptomycin-resistant and either methanol⁺ or methanol⁻. The frequencies of streptomycin-resistant transformants and methanol⁺ transformants were determined independently. Four of the methanol⁻ mutant strains appeared to contain mutations which are linked to each other (group 1) while the other four (group 2) appeared to contain mutations not linked to the first four. In reciprocal crosses with the group 2 markers, two appeared to be linked to each other, while the other two were not linked to any mutant tested.

The data presented were used to construct a model for the organization of these genes in which the CO-binding cytochrome *c* is not linked to the five genes for C₁-specific enzymes. In this model, the expression of six C₁-specific functions is controlled by a common regulatory agent.

INTRODUCTION

Several enzymes responsible for carbon assimilation during growth on C₁ compounds are coordinately expressed in the facultative methanol-utilizing bacterium, *Pseudomonas* AM1, and the facultative methane-utilizing bacterium, *Methylobacterium organophilum* (Large & Quayle, 1963; Dunstan, Anthony & Drabble, 1972; O'Connor & Hanson, 1977). In *M. organophilum*, the ability to oxidize methane and the synthesis of intracytoplasmic membranes occur only in methane-grown cells (Patt *et al.*, 1974, 1977). In the past, co-ordinate expression of enzymes in response to the substrate they act upon has been an indication of control by a regulon or operon (Jacob & Monod, 1961*a, b*; Buttin, 1962; Englesberg *et al.*, 1965; Power, 1967; Ames, Garry & Herzenberg, 1960; Lester & Yanofsky, 1961).

Several mutants have been isolated in *M. organophilum* which are pleiotropically negative for several enzymes involved in the utilization of C₁ compounds (O'Connor & Hanson, 1977). Pleiotropy in other bacteria has been explained by mutations in an operon resulting from deletions, polar nonsense or frameshift mutations, or mutations in regulatory genes (Goldberger & Berberich, 1966; Yanofsky & Ito, 1966; Cohn & Monod, 1953).

The existence of an operon for the genes specifying enzymes involved in the utilization of

C₁ compounds in *M. organophilum* can be verified by genetic mapping. This paper describes the determination of genetic linkage relationships between C₁ mutants of *M. organophilum*, using genetic transformation as a mapping tool.

METHODS

Organism. *Methylobacterium organophilum* (ATCC27886, Patt, Cole & Hanson, 1976) was used in all experiments.

Media and growth conditions. The modified nitrate mineral salts medium (NMS) previously described (Patt *et al.*, 1976) was used for all studies. Succinate and yeast extract were dissolved in distilled water, sterilized separately and added directly to the growth medium at concentrations of 0.1 % (w/v) and 0.05 % (w/v) respectively. Methanol (96 %, v/v) and streptomycin were filter-sterilized and added separately to the growth medium at concentrations of 0.5 % (v/v) and 0.005 % (w/v), respectively. All cultures were incubated at 30 °C. Liquid cultures were incubated on rotary shakers.

Preparation of extracts. Extracts used for enzyme assays were prepared as described previously (O'Connor & Hanson, 1975). Protein was estimated by the method of Lowry *et al.* (1951).

Enzyme activities. Enzymes were assayed as described previously (O'Connor & Hanson, 1977).

Cytochrome detection. Cytochrome was detected on polyacrylamide gels using the gel buffer system and the staining method described by Clarke (1964). Samples containing 200 ± 20 µg protein were added to each gel.

Mutant isolation. Mutants unable to grow on C₁ compounds were isolated using the ultraviolet light procedure described previously (O'Connor, Wopat & Hanson, 1977).

Reversion tests. Revertants were obtained using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) and ethyl methanesulphonate (EMS) as described previously (O'Connor & Hanson, 1977).

DNA isolation. DNA was isolated as described previously (O'Connor *et al.*, 1977).

Transformation. Transformation experiments were carried out using the method described previously (O'Connor *et al.*, 1977). DNA (50 µg ml⁻¹) was incubated with the bacteria during growth. Cultures containing 0.1 × SSC (SSC: 0.15 M-sodium chloride/0.015 M-sodium citrate, pH 7.3) in place of DNA were included as controls for reversion with each experiment. The total number of recipient bacteria was determined by total viable counts on NMS medium plus yeast extract and succinate or methanol as the carbon and energy sources. Streptomycin-resistant (Sm^R) transformants were detected using an overlay technique. Plates of NMS media with the appropriate carbon and energy source were spread with potential transformants and incubated overnight at 30 °C; 2.5 ml of 0.6 % (w/v) agar containing 100 µg streptomycin ml⁻¹ was then layered on top of the bottom agar. The streptomycin-resistant clones were scored after 7 days incubation at 30 °C.

Strain construction. Streptomycin-resistant mutants of isolates 8Z and 4M were prepared by transformation, using DNA from a streptomycin-resistant strain (O'Connor *et al.*, 1977).

Antiserum. Antiserum was prepared in rabbits against purified methanol dehydrogenase (H. Wolf & R.S. Hanson, unpublished results). Two injections of 1 mg each of purified methanol dehydrogenase (from *M. organophilum*) incorporated in Freund's complete adjuvant were given intramuscularly to random-bred New Zealand rabbits at 1 week intervals. Two weeks later, three intravenous injections of 0.2 mg soluble antigen each were given at 2 day intervals. The rabbits were bled by marginal ear puncture 1 week later and at intervals during the following weeks. From the blood obtained, serum was prepared, pooled, titred and stored at -20 °C. When necessary, booster doses of 1 mg of enzyme in Freund's complete adjuvant were given intramuscularly to increase the titre.

Cross-reactivity was determined by the Ouchterlony double diffusion technique (Ouchterlony, 1949). Reactions were carried out at 20 °C and interpreted after 24 h incubation.

RESULTS

Mutants

The mutants used in this study are described in Table 1. All were unable to grow on methanol, but were able to grow on NMS medium with succinate and yeast extract as the carbon and energy sources. All mutants were routinely grown on media containing succinate plus yeast extract. For enzyme studies, all strains were grown on media containing succinate plus yeast extract plus methanol. The enzyme activities in mutants 8Z, 17M, 4M, 7A, 17A and 8A were published previously (O'Connor & Hanson, 1977).

The properties of methanol-dependent cytochromes in *Pseudomonas* AM1 have been

Table 1. *Phenotypes of mutants and wild-type M. organophilum*

Cultures were grown on media containing 0.1 % succinate, 0.5 % methanol and 0.05 % yeast extract. Enzyme activities are expressed as $\mu\text{mol min}^{-1} (\text{mg protein})^{-1}$ and are the average of two or more experiments. Values agreed within $\pm 20\%$.

Phenotype	Wild type	Single mutants				Pleiotropic mutants			
		8Z	17M	22C	24C	4M	7A	17A	8A
Hydroxypyruvate reductase	0.38	0.27	0.28	0.33	0.26	0.03	ND	0.03	0.05
Serine-glyoxylate aminotransferase	0.11	0.08	0.08	0.08	0.09	0.01	NA	0.001	0.03
Methanol dehydrogenase	0.08	0.09	ND	0.04	0.07	ND	ND	ND	ND
Glycerate kinase	0.07	0.006	0.06	0.05	0.06	0.01	0.10	0.02	0.06
Serine transhydroxymethylase									
+ glyoxylate*	0.16	0.12	0.19	0.12	0.14	0.08	0.01	0.03	0.20
- glyoxylate	0.13	0.07	0.16	0.07	0.07	0.10	0.01	0.03	0.01
Major cytochrome bands									
<i>R_m</i>	0.48	0.49	0.45	0.45	0.20†	0.49	0.44	0.45	0.40
Intensity	4+	4+	4+	4+	4+	+/-	4+	4+	+/-
Cross-reacting material‡	+	+	-	+	+	-	-	-	-
Revertibility by NTG and EMS		+	-	-	+	+	-	-	+

ND, Not detected; NA, not assayed.

* Assayed in the presence of 5 mM-glyoxylate which stimulates the activity of the isoenzyme predominant in cells grown on methanol (O'Connor & Hanson, 1975).

† The cytochrome band with an *R_m* of 0.20 was not detected in other strains.

‡ Antisera prepared against purified methanol dehydrogenase.

described by Anthony (1975) and Widdowson & Anthony (1975). A mutant in the facultative methanol-oxidizing bacterium, *Pseudomonas* AM1, which lacks cytochrome *c* is unable to grow on methanol (Anthony, 1975). Tonge and co-workers (1975) have discussed a possible role for cytochrome *c* in *Methylosinus trichosporium*. The cytochrome *c* from *M. organophilum* resembles that from *Pseudomonas* AM1 in spectral properties (oxidized minus reduced and CO minus reduced difference spectra) as well as having a similar molecular weight (H. Wolf & R. S. Hanson, unpublished results).

A cytochrome which appears identical to cytochrome *c* from *Pseudomonas* AM1 was readily detected using the gel system developed by Clarke (1964) in which haem proteins were detected by staining polyacrylamide gels with benzidine reagent. This cytochrome occurred as the only dark-staining band in extracts of wild-type cells grown on methanol or methanol plus succinate and had a relative mobility of 0.48. Other haem proteins occurred as very light-staining bands on these gels. A purified preparation of cytochrome *c* from *M. organophilum* had a relative mobility of 0.48 and was found only in cells grown in the presence of methanol (J. Bishop, H. Wolf, M. O'Connor & R. S. Hanson, unpublished results). The cytochrome detection system was not quantified, but identical gels containing differing amounts of protein showed corresponding differences in the intensity of the cytochrome band.

Mutant strain 8Z had low levels of glycerate kinase (EC 2.7.1.31), mutant 17M lacked methanol dehydrogenase (EC 1.1.99.8), and mutant 24C had low levels of cytochrome *c*. Mutant 22C had a second intense cytochrome band not seen in the wild type, and had lower levels of methanol dehydrogenase than the wild-type strain. The other four mutants lacked methanol dehydrogenase activity and had low levels of two or more other enzymes. Mutants 8Z, 24C, 4M and 8A were revertible to the methanol⁺ phenotype by NTG and EMS, while the other four isolates were not. Enzyme levels were not tested in these revertants. All mutants which lacked methanol dehydrogenase activity, also lacked cross-reacting material to antiserum prepared against purified methanol dehydrogenase.

Table 2. Transformation frequencies for crosses involving methanol⁻ recipient cells and DNA from streptomycin-resistant (Sm^R) cells which were either methanol⁺ or methanol⁻ (strain 8Z)

Frequencies are expressed as the ratio of the number of methanol⁺ or streptomycin-resistant recombinants to the total number of recipient cells. All controls containing no DNA had frequencies of less than 10⁻⁷.

Recipient	Methanol ⁺ /total		Sm ^R /total	
	8Z Sm ^R DNA	Sm ^R DNA	8Z Sm ^R DNA	Sm ^R DNA
4M	< 10 ⁻⁷	1.1 × 10 ⁻⁶	4.0 × 10 ⁻⁵	3.5 × 10 ⁻⁶
	< 10 ⁻⁷	6.5 × 10 ⁻⁶	6.0 × 10 ⁻⁶	5.2 × 10 ⁻⁵
7A	< 10 ⁻⁷	6.0 × 10 ⁻⁵	1.2 × 10 ⁻⁵	3.0 × 10 ⁻⁵
	< 10 ⁻⁷	7.4 × 10 ⁻⁵	4.6 × 10 ⁻⁵	3.1 × 10 ⁻⁵
17M	< 10 ⁻⁷	3.3 × 10 ⁻⁵	1.0 × 10 ⁻⁶	7.7 × 10 ⁻⁶
	< 10 ⁻⁷	1.0 × 10 ⁻⁵	6.0 × 10 ⁻⁶	1.3 × 10 ⁻⁵
8Z	< 10 ⁻⁷	1.0 × 10 ⁻⁶	1.2 × 10 ⁻⁵	2.5 × 10 ⁻⁵
24C	7.5 × 10 ⁻⁶	1.0 × 10 ⁻⁵	4.0 × 10 ⁻⁶	6.2 × 10 ⁻⁶
	2.8 × 10 ⁻⁵	6.6 × 10 ⁻⁶	5.0 × 10 ⁻⁵	3.0 × 10 ⁻⁵
22C	6.0 × 10 ⁻⁶	2.0 × 10 ⁻⁵	3.2 × 10 ⁻⁶	1.4 × 10 ⁻⁵
	1.0 × 10 ⁻⁶	1.4 × 10 ⁻⁵	1.0 × 10 ⁻⁵	6.0 × 10 ⁻⁵
17A	7.5 × 10 ⁻⁵	7.5 × 10 ⁻⁵	8.0 × 10 ⁻⁵	5.0 × 10 ⁻⁶
	1.0 × 10 ⁻⁶	7.5 × 10 ⁻⁶	2.0 × 10 ⁻⁵	5.0 × 10 ⁻⁵
8A	2.5 × 10 ⁻⁶	2.0 × 10 ⁻⁵	5.6 × 10 ⁻⁵	1.7 × 10 ⁻⁶
	8.5 × 10 ⁻⁶	8.3 × 10 ⁻⁶	1.2 × 10 ⁻⁶	6.7 × 10 ⁻⁶

Table 3. Transformation frequencies for crosses involving methanol⁻ recipient cells and DNA from streptomycin-resistant cells which were either methanol⁺ or methanol⁻ (strain 4M)

Frequencies are expressed as the ratio of the number of methanol⁺ or streptomycin-resistant recombinants to the total number of recipient cells. All controls containing no DNA had frequencies of less than 10⁻⁷.

Recipient	Methanol ⁺ /total		Sm ^R /total	
	4M Sm ^R DNA	Sm ^R DNA	4M Sm ^R DNA	Sm ^R DNA
4M	< 10 ⁻⁷	4.0 × 10 ⁻⁶	7.2 × 10 ⁻⁶	2.8 × 10 ⁻⁵
7A	< 10 ⁻⁷	1.6 × 10 ⁻⁵	1.2 × 10 ⁻⁵	7.5 × 10 ⁻⁶
17M	< 10 ⁻⁷	1.3 × 10 ⁻⁵	3.0 × 10 ⁻⁵	2.2 × 10 ⁻⁵
8Z	< 10 ⁻⁷	4.4 × 10 ⁻⁶	1.5 × 10 ⁻⁵	4.0 × 10 ⁻⁵
24C	1.2 × 10 ⁻⁶	1.7 × 10 ⁻⁵	3.7 × 10 ⁻⁶	5.0 × 10 ⁻⁶
22C	1.9 × 10 ⁻⁵	3.0 × 10 ⁻⁵	2.3 × 10 ⁻⁵	1.4 × 10 ⁻⁵
17A	1.0 × 10 ⁻⁶	6.3 × 10 ⁻⁵	2.3 × 10 ⁻⁵	6.7 × 10 ⁻⁶
8A	3.0 × 10 ⁻⁵	4.0 × 10 ⁻⁶	7.7 × 10 ⁻⁶	2.0 × 10 ⁻⁵

Strain construction

Attempts were made to transform methanol⁻ markers from 17M and 8Z into the highly transformable glutamate auxotroph described previously (O'Connor *et al.*, 1977). Transformation frequencies of 10⁻³ were expected. However, although over 20000 colonies were examined, no methanol⁻ glutamate⁻ mutants were isolated. Streptomycin-resistant methanol⁻ mutants were constructed by adding DNA from a streptomycin-resistant strain to methanol⁻ recipient strains 8Z and 4M. Transformation frequencies were approximately 10⁻⁵.

Linkage relationships

DNA preparations from the streptomycin-resistant mutants of strains 8Z and 4M were used in transformation studies to determine linkage relationships with the eight methanol⁻ mutants. Each mutant was incubated with DNA from streptomycin-resistant methanol⁻ cells, with DNA from streptomycin-resistant methanol⁺ cells, or without DNA. The number of methanol⁺ transformants and the number of streptomycin-resistant transformants were determined independently in each cross.

Table 4. Transformation frequencies for crosses involving methanol⁻ recipient cells and DNA from methanol⁺ or methanol⁻ cells

Frequencies are expressed as the ratio of the number of methanol⁺ recombinants to the total number of recipient cells. All controls containing no DNA had frequencies of less than 10⁻⁷.

Donor DNA	Recipient		
	8A	17A	24C
8A	ND	6.7×10^{-5}	2.3×10^{-5}
22C	8.0×10^{-6}	2.5×10^{-5}	$< 10^{-7}$
24C	7.5×10^{-5}	8.0×10^{-5}	ND
Wild type	2.9×10^{-5}	1.0×10^{-5}	5.0×10^{-6}

ND, Not done.

The results of the crosses are presented in Tables 2 and 3. In each cross, streptomycin-resistant and methanol⁺ transformants were obtained when DNA from a streptomycin-resistant methanol⁺ strain was used. However, when DNA from streptomycin-resistant mutants of methanol⁻ strains 8Z or 4M was used, streptomycin-resistant transformants were obtained but no methanol⁺ transformants were found when mutant strains 4M, 7A, 17M or 8Z were used as recipients. The other four mutants (22C, 24C, 8A and 17A) yielded methanol⁺ recombinants with DNA from both methanol⁻ and methanol⁺ strains.

Reciprocal crosses between mutants 22C, 24C, 8A and 17A produced methanol⁺ recombinants in all cases except that of 24C crossed with 22C (Table 4).

Reversion of a streptomycin-resistant mutant of isolate 4M

A streptomycin-resistant mutant of isolate 4M was reverted to a streptomycin-resistant methanol⁺ phenotype using EMS. Activities of glycerate kinase, serine transhydroxymethylase, serine-glyoxylate aminotransferase, hydroxypyruvate reductase and methanol dehydrogenase were at partially constitutive levels in cells of this revertant grown on succinate. These levels were 20 to 40 % lower than those in wild-type cells grown on methanol plus succinate. The level of cytochrome *c* was similar to that found in wild-type cells grown on succinate.

DISCUSSION

Evidence is presented which suggests genetic linkage for some *C*₁-specific functions in *M. organophilum*. Non-detectable transformation frequencies in crosses between methanol⁻ mutants suggest there is genetic linkage between the two markers tested. The transfer of a streptomycin-resistance marker indicated that the cells in each cross were competent, while the transfer of methanol⁺ markers into methanol⁻ mutants showed that each mutant was capable of being transformed to the methanol⁺ phenotype.

Isolates 8Z and 4M were shown to be linked in reciprocal crosses, and each was found to be linked to 17M and 7A. Crosses between donor strains 8Z and 4M and recipient strains 8A, 17A, 22C and 24C resulted in methanol⁺ transformants at frequencies approximately equal to those obtained with wild-type DNA. In addition, reciprocal crosses between these four markers indicate that 22C and 24C are linked only to each other, and 8A and 17A are not linked to any of the markers tested.

The transformation frequencies obtained varied over a 10-fold range, and the data were not adequate for fine-structure mapping. However, the linkage relationships outlined above, combined with the characteristics of the mutants, allow the formulation of a plausible model which is consistent with all the data (Fig. 1).

Mutant 24C appears to be a point mutation in a gene required for production of cytochrome *c*. Mutant 22C appears to produce an altered cytochrome. Isolates 22C and 24C are

Fig. 1. Model for the arrangement of genes coding for C_1 -specific functions in *Methylobacterium organophilum*.

The mutant phenotypes were: 17M, non-revertible MeDH⁻; 8Z, revertible GlycKin^{low}; 4M, revertible HPred^{low} SGAT^{low} GlycKin^{low} STHM⁻ MeDH⁻; 17A, non-revertible HPred^{low} SGAT^{low} GlycKin^{low} STHM⁻ MeDH⁻ Cyt^{low}; 7A, non-revertible HPred⁻ SGAT⁻ STHM⁻ MeDH⁻; 24C, revertible Cyt^{low}; 22C, non-revertible extra Cyt band; 8A, revertible HPred^{low} SGAT^{low} MeDH⁻.

GlycKin, Glycerate kinase; HPred, hydroxypyruvate reductase; SGAT, serine-glyoxylate aminotransferase; Cyt, cytochrome *c*; STHM, serine transhydroxymethylase (glyoxylate activating); MeDH, methanol dehydrogenase; low, detectable but less than 25% of wild-type activity; -, not detectable; p, p', promoter; o, operator; R, regulatory agent.

unlinked to all other mutants tested, but are linked to each other. Cytochrome *c* is induced by methanol, and it is assumed that a common regulatory agent (R) is involved in controlling both sets of genes.

Mutant 17A is a non-revertible mutant with lowered levels of all C_1 -specific enzymes tested and cytochrome *c*. It is not linked to any of the other mutants. There is not sufficient data to determine whether the proposed regulatory agent is a protein or a low molecular weight molecule, or whether it acts in a positive or negative manner.

Mutants 4M, 8Z, 7A and 17M all contain mutations which are linked to each other. Therefore, in the model they are placed adjacent to one another. Isolate 4M contains lowered levels of the five C_1 -specific enzymes, but not cytochrome *c*, and it is revertible. One revertant of isolate 4M was partially constitutive for the five C_1 -specific enzymes. This supports the possibility that the lesion in 4M may involve a regulatory component, possibly an operator or promoter. This information does not rule out either positive or negative control in this system, since partially constitutive revertants of regulatory mutants have been described in both positive (Gielow, Lagen & Englesberg, 1971) and negative (Arditti, Scaife & Beckwith, 1968) control systems. Mutant 8Z has a revertible mutation in glycerate kinase. Mutant 7A contains a non-revertible mutation which results in no detectable activity of four of the C_1 -specific enzymes. It should be noted that the assay for serine-glyoxylate aminotransferase (EC 2.6.1.45) is coupled to hydroxypyruvate reductase (EC 1.1.1.29). Therefore, when there is no detectable hydroxypyruvate reductase activity, serine-glyoxylate aminotransferase cannot be assayed. At present, there is no source of hydroxypyruvate reductase free of serine-glyoxylate aminotransferase activity available to us that could be used for this assay. Isolate 7A is assumed to be a deletion, and is placed distal to 8Z, since it has wild-type levels of glycerate kinase. Mutation 17M is positioned at the end of the operon because it is a non-revertible mutation which affects only methanol dehydrogenase.

The deficiencies in hydroxypyruvate reductase, serine transhydroxymethylase (EC 2.1.2.1) and serine-glyoxylate aminotransferase in mutant 7A might result from defects in structural genes or defects in one or more genes regulating the synthesis of these three enzymes.

Isolate 8A also appears to be a regulatory mutant. It is revertible and unlinked to the other mutants and affects the synthesis of at least three C_1 -specific enzymes. Since there was no detectable cross-reacting material for methanol dehydrogenase in this mutant, the mutation probably does not affect a component which interacts with the intact enzyme molecules. The low level of serine transhydroxymethylase in the absence of glyoxylate for isolate 8A might suggest that the cellular pool of glyoxylate is very low in this mutant. The level of

serine transhydroxymethylase in the presence of glyoxylate is similar to that in the wild type.

The inducibility of all other C_1 -specific enzymes in a mutant lacking methanol dehydrogenase (mutant 17M) suggests that the inducer in this system is either methanol, or some compound derived from methanol via an unknown pathway unaffected by the absence of methanol dehydrogenase.

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REFERENCES

- AMES, B. N., GARRY, B. & HERZENBERG, L. A. (1960). The genetic control of the enzymes of histidine biosynthesis in *Salmonella typhimurium*. *Journal of General Microbiology* **22**, 369-375.
- ANTHONY, C. (1975). The microbial metabolism of C_1 compounds. The cytochromes of *Pseudomonas* AM1. *Biochemical Journal* **146**, 289-298.
- ARDITTI, R. R., SCAIFE, J. & BECKWITH, J. R. (1968). The nature of mutants in the LAC promoter region. *Journal of Molecular Biology* **38**, 421-426.
- BUTTIN, G. (1962). Sur la structure de l'opéron galactose chez *E. coli* K-12. *Comptes rendus hebdomadaire des Séances de l'Académie des sciences* **255**, 1233-1241.
- CLARKE, J. T. (1964). Simplified 'disc' (polyacrylamide gel) electrophoresis. *Annals of the New York Academy of Sciences* **121**, 428-444.
- COHN, M. & MONOD, J. (1953). Specific inhibition and induction of enzyme synthesis. *Symposia of the Society for General Microbiology* **3**, 132-140.
- DUNSTAN, P. M., ANTHONY, C. & DRABBLE, W. T. (1972). Microbial metabolism of C_1 and C_2 compounds. The role of glyoxylate, glycollate and acetate in the growth of *Pseudomonas* AM1 on ethanol and on C_1 compounds. *Biochemical Journal* **128**, 107-115.
- ENGLESBERG, E., IRR, J., POWER, J. & LEE, N. (1965). Positive control of enzyme synthesis by gene C in the L-arabinose system. *Journal of Bacteriology* **90**, 946-955.
- GIELOW, L., LARGEN, M. & ENGLESBERG, E. (1971). Initiator constitutive mutants of the L-arabinose operon (OI-BAD) of *Escherichia coli* B/r. *Genetics* **69**, 289-302.
- GOLDBERGER, R. R. & BERBERICH, M. A. (1966). Studies on the mechanism of derepression of the histidine operon in *S. typhimurium*. *Federation Proceedings* **25**, 337-338.
- JACOB, F. & MONOD, J. (1961a). Genetic regulatory mechanisms in the synthesis of proteins. *Journal of Molecular Biology* **3**, 318-325.
- JACOB, F. & MONOD, J. (1961b). On the regulation of gene activity. *Cold Spring Harbor Symposia on Quantitative Biology* **26**, 193-210.
- LARGE, P. J. & QUAYLE, J. R. (1963). Microbial growth on C_1 compounds. 5. Enzyme activities in extracts of *Pseudomonas* AM1. *Biochemical Journal* **87**, 386-396.
- LESTER, G. & YANOFSKY, C. (1961). Influence of 3-methylanthranilic acids on the formation of tryptophan synthetase in *Escherichia coli*. *Journal of Bacteriology* **81**, 81-95.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265-275.
- O'CONNOR, M. L. & HANSON, R. S. (1975). Serine transhydroxymethylase isoenzymes from a facultative methylotroph. *Journal of Bacteriology* **124**, 985-996.
- O'CONNOR, M. L. & HANSON, R. S. (1977). Enzyme regulation in *Methylobacterium organophilum*. *Journal of General Microbiology* **101**, 327-332.
- O'CONNOR, M. L., WOPAT, A. E. & HANSON, R. S. (1977). Genetic transformation in *Methylobacterium organophilum*. *Journal of General Microbiology* **98**, 265-272.
- OUCHTERLONY, O. (1949). Antigen-antibody reactions in gels. *Acta pathologica et microbiologica scandinavica* **26**, 507-515.
- PATT, T. E., COLE, G. C., BLAND, J. & HANSON, R. S. (1974). Isolation and characterization of bacteria that grow on methane and organic compounds as sole sources of carbon and energy. *Journal of Bacteriology* **120**, 955-964.
- PATT, T. E., COLE, G. C. & HANSON, R. S. (1976). *Methylobacterium*, a new genus of facultatively methylotrophic bacteria. *International Journal of Systematic Bacteriology* **26**, 226-229.
- PATT, T. E., O'CONNOR, M. L., COLE, G. C., DAY, R. & HANSON, R. S. (1977). Characterization of a facultative methylotrophic bacterium. In *Microbial Production and Utilization of Gases*, pp. 317-327. Edited by H. G. Schlegel, G. Gottschalk and N. Pfennig. Göttingen: Akademie der Wissenschaften zu Göttingen.
- POWER, J. (1967). The L-rhamnose genetic system in *Escherichia coli* K-12. *Genetics* **55**, 557-568.
- TONGE, G. M., HARRISON, D. E. F., KNOWLES, C. J. & HIGGINS, I. J. (1975). Properties and partial purification of the methane-mono-oxygenase enzyme system from *Methylosinus trichosporium*. *FEBS Letters* **58**, 293-299.
- WIDDOWSON, D. & ANTHONY, C. (1975). The microbial metabolism of C_1 compounds. The electron transport chain of *Pseudomonas* AM1. *Biochemical Journal* **152**, 349-356.
- YANOFSKY, C. & ITO, J. (1966). Nonsense codons and polarity on the tryptophan operon. *Journal of Molecular Biology* **21**, 313-323.