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Comparison of Nitrate Reductase Mutants of *Escherichia coli* Selected by Alternative Procedures

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Summary. Seventy-two mutants deficient in formate-nitrate reductase activity were selected in *Escherichia coli* strain PK 27, by two different procedures. Forty-five strains were selected on the basis of chlorate resistance and 27 strains were selected by their inability to reduce nitrate with formate as an electron donor. Genetic analysis of these strains showed that the two techniques yield distinctly different distributions of mutants among the various controlling genetic loci. Chlorate resistance appears to select for severe alterations in the nitrate reductase system; 98% of these mutants fell into the pleiotropic *chl* A, B, D and E classes and are deficient in all the activities of the formate-hydrogenlyase pathway as well as formate-nitrate reductase pathway. In contrast, 48% of the mutants selected for their inability to reduce nitrate with formate as the electron donor were of the *chl* C class and two new classes were identified among mutants selected by this procedure. *Chl* F mutants are linked to tryptophan and the *chl* C locus. *Chl* G mutants map at zero minutes on the *E. coli* genetic map.

Introduction

Under anaerobic growth conditions *Escherichia coli* metabolizes formate by either of two pathways. In the presence of nitrate, a membrane-bound formate-nitrate reductase complex is induced which oxidizes formate to CO_2 and reduces nitrate to nitrite (Iida and Taniguchi, 1959; Ruiz-Herrera and DeMoss, 1969; Wimpenny and Cole, 1967). In the absence of nitrate, *E. coli* forms the formate-hydrogenlyase system which converts formate to carbon dioxide and hydrogen (Gray and Gest, 1965; Peck and Gest, 1956). The relatively simple nitrate reductase complex provides a very promising system for asking general questions about the regulation, synthesis and organization of multi-component membrane-bound complexes. One approach to these questions is to consider the amount of genetic information that is necessary for the expression of the formate-nitrate reductase pathway.

Mutants defective in the nitrate reductase system have been selected by several techniques and each appears to yield an entirely different spectrum of mutants. Mutants selected by resistance to chlorate have been designated either *chl* or *nar* and shown to map at five distinct positions on the *E. coli* chromosome (Fig. 1; *chl* A, B, C, D and E) (Puig *et al.*, 1967; Adyha *et al.*, 1968; Venables and Gest, 1968). *Chl* A, B, D and E mutants are pleiotropic in that they lack the activities of the formate-nitrate reductase pathway as well as the formate-hydrogenlyase pathway. Guest (1969) has shown that *chl* C mutations affect only the formate-nitrate reductase system and has proposed that the *chl* C locus is the structural gene for nitrate reductase. According to Casse (1970), 93% of spontaneous Chl^r

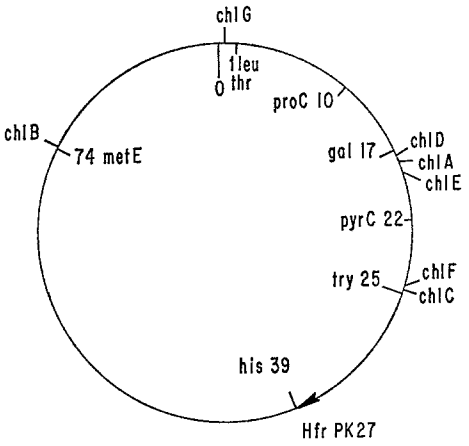

Fig. 1. Map of *chl* mutants on the *E. coli* chromosome. The numbers indicate the map position of the indicated markers in minutes

mutants are linked to the gal-bio region. This would include the *chl* A, D and E mutants. Six per cent are *chl* B and 1% are *chl* C.

Ruiz-Herrera *et al.* (1969) have described another technique for obtaining nitrate reductase mutants which depends on a direct screening of colonies for their inability to utilize formate as an electron donor for nitrate reduction. Mutants recovered by this procedure, designated NR^-, appeared to differ both biochemically and genetically from the chlorate resistant mutants selected by other workers. Biochemically they fell into six classes having defects in formate dehydrogenase or nitrate reductase or combinations of these and cytochrome b_1. Genetic analysis by Hfr conjugation showed that most, if not all of these mutants were linked to tryptophan (Ruiz-Herrera *et al.*, 1969). More recent studies have shown that only those mutants defective in nitrate reductase or nitrate reductase and cytochrome b_1 are linked to tryptophan by P1 transduction (Glaser and DeMoss, unpublished results). Nevertheless, this class still represents 50% of the original isolates.

The mutants previously selected by the two techniques were selected in different strains of *E. coli*. The apparent difference in the spectrum of mutants obtained by chlorate resistance and those obtained by screening for loss of formate-dependent nitrate reduction led us to select new mutants by both techniques in the same genetic background and to carefully compare the frequency and distribution of the different mutant types.

Materials and Methods

The strains used in this study which are listed in Table 1 were maintained on nutrient agar slants.

Selection of Mutants. Chlorate resistant mutants were selected from nitrosoguanidine mutagenized cells of PK 27 as described previously (Glaser and DeMoss, 1971). For selection of mutants unable to carry out a formate-dependent nitrate reduction, logarithmically growing cells of PK 27 were treated with nitrosoguanidine according to the procedure of Adelberg *et al.* (1965), diluted and planted on L-agar (Lennox, 1955) containing 0.01%

potassium nitrate. For best results, it was important to keep the number of colonies per plate between 75 and 150. The colonies were replica plated to another L-agar plate and the original plate overlayed with 5 ml of soft agar (0.75%) containing the reagents for the formate-nitrate reductase assay: 0.1 M potassium phosphate, pH 7.0, 0.5 M sodium formate, 1% potassium nitrate. After incubation at room temperature for 15 minutes the plate was overlayed with an additional 5 ml of soft agar containing the reagents for nitrite determination. It was necessary to mix equal volumes of molten agar (1.5%) and nitrite color reagent (2 parts 4% sulfanilamide in 25% concentrated HCl: 1 part 0.08% N-1-naphthylethylenediamine dihydrochloride) just prior to use. Mutant colonies failed to produce the purple halo due to nitrite accumulation.

All mutants were tested for their ability to grow on minimal-glucose medium containing 50 μg/ml of thiamine, for their resistance to chlorate and for their ability to form gas. Chlorate resistance was tested by stabbing the mutant strain into 2 ml of L-agar containing 0.5% potassium chlorate in a 13×100 mm test tube and overlaying with 2.5 ml of the same agar. Growth was scored as compared to wild-type PK 27 at 12, 24 and 48 hours. A crude estimation of gas forming ability was obtained in stabs of the same type using either L-agar or minimal-glucose medium supplemented with 10^{-6} M sodium molybdate and 10^{-6} M sodium selenite (Lester and DeMoss, 1971). If the strain produced the formate-hydrogenlyase system, within 24 hours the top layer of agar was forced up the tube as a result of gas evolution.

Genetic Techniques

Conjugation. All formate-nitrate reductase mutants were selected in the Hfr strain PK 27. When this strain is mated with an F^- strain, chromosome transfer begins at the histidine marker and proceeds in a counterclockwise direction (Fig. 1) (Kahn, 1968). Both mating types were grown in L-broth into log phase and 5 ml amounts were mixed. Mating proceeded for 90 minutes at 37°C. The cells were then centrifuged, resuspended in 1 ml of sterile saline and appropriately diluted into sterile saline before plating on the selective medium.

Transduction. The procedure of Lennox (1955) was utilized to transduce with phage P1.

Scoring Colonies for Formate-Nitrate Reductase Activity

Colonies to be scored for formate-nitrate reductase activity were stabbed into an L-agar petri plate containing 0.01% potassium nitrate and incubated for 8 to 12 hours. Forty-four colonies could be stabbed per plate. The plates were then overlayed with 5 ml of soft agar (0.75%) containing the reagents of the formate-nitrate reductase assay. After 15 minutes incubation at room temperature the plates were overlayed with an additional 5 ml of soft agar containing the color reagents for nitrite determination. Those colonies which can reduce nitrate to nitrite produce a purple halo.

Results

A total of 72 mutants were selected in the Hfr strain PK 27 using the procedures described in Methods. Forty five mutants (Chl^r) were selected by their resistance to chlorate and 27 mutants (NR^-) were selected by their inability to reduce nitrate to nitrite with formate as an electron donor. All mutants isolated by the two procedures possessed the NR^- phenotype. Therefore, throughout this paper both NR^- and Chl^r are used to indicate the phenotype of strains which are unable to carry out a formate-dependent reduction of nitrate. The genetic loci at which these various phenotypes map are referred to as *chl* loci.

An initial genetic analysis was performed by mating each of the mutant strains with the F^- strain SA 291 which carries a deletion that extends from *aro* G through *chl* A (Fig. 2). The Str-r F^- strain was Gal^- and NR^- by virtue of the fact that the deletion includes *gal* and both the *chl* A and *chl* D genes. The Str-s Hfr mutants were Gal^+ and NR^-. Gal^+ recombinants were selected on

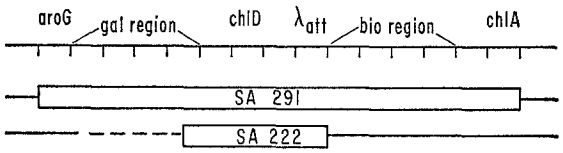

Fig. 2. Map of the deletion strains SA 291 and SA 222. The blocked area represents the extent of the deletion for each strain

Table 1. Strains of *E. coli* used in this study

Strain	Phenotype[a]	Source
PK 27	Hfr Thi⁻ Str-s	D. Helinski (Kahn, 1968)
AT 3143 (CGSC no. 4539)[b]	F⁻ PyrC⁻ Ilv⁻ Met⁺ His⁻ PurE⁻ ProC⁻ proC⁻ PdxC⁻ Cyc-r Xyl⁻ Lac⁻ Str-r Tsx-r	Coli Genetic Stock Center[c] (Taylor, 1970)
AB 2102	F⁻ Str-r Thi⁻ Leu⁻ Thr⁻ Pro⁻ Lac⁻ T6-r Gal⁻ Trp⁻ His⁻ Mal⁻ Mtl⁻	D. Helinski
X997 (CGSC no. 3598)[b]	F⁻ pyr C⁻ Ilv⁻ ArgF⁻ Met⁻ His⁻ Trp⁻ Pur E⁻ Pro C⁻ Thr⁻ Tsx-r Tsm	Coli Genetic Stock Center[c]
SA 291	F⁻ Gal⁻ Bio⁻ Aro G⁻ His⁻ Chl-r Str-r	A. Campbell (Adhya *et al.*, 1968)
SA 222	F⁻ Gal⁻ Chl-r Str-r	A. Campbell (Adhya *et al.*, 1968)

[a] The nomenclature used in describing the strains is that given by Taylor (1970).
[b] Coli Genetic Stock Center strain number.
[c] Coli Genetic Stock Center, Department of Microbiology, Yale University School of Medicine, 310 Cedar Street, New Haven, Connecticut 06510.

minimal salts medium supplemented with 1% galactose and 200 μg/ml of streptomycin sulfate, and scored for their ability to carry out the formate-dependent reduction of nitrate as described in Methods.

Mutants which failed to give any Gal⁺ NR⁺ recombinants were assumed to fall within the deleted portion of the F⁻ chromosome and therefore assigned to the *chl* A or *chl* D classes (Table 2). These two classes were distinguished on the basis of conjugation to a second, shorter deletion strain. The deletion SA 222 covers only the *gal* to *chl* D region of the genome (Fig. 2) and those mutants which showed between 1% and 8% Gal⁺ NR⁺ recombinants were classified as *chl* A mutants (Table 2). Although *chl* D mutants failed to recombine with either SA 291 or SA 222 to give Gal⁺ NR⁺ recombinants, these strains nevertheless represent a homogeneous class since all mutants identified as *chl* D were phenotypically restored to wild-type by the addition of 10^{-4} M molybdate to the growth medium (Glaser and DeMoss, 1971).

Mutants were assigned to the *chl* E class on the basis of a low level of NR⁺ Gal⁺ recombinants (5% to 13%) with strain SA 291. This level of recombination

Table 2. Deletion Mapping of Nitrate Reductase Mutants. Hfr strains: PK 27 Gal$^+$ Str-s Chl^r (NR$^-$). F$^-$ strains: SA 291 Gal$^-$ Str-r Chl^r (NR$^-$), SA 222 Gal$^-$ Str-r Chl^r (NR$^-$)

Selective procedure	Number of mutants	Frequency of NR$^+$ among Gal$^+$ recombinants		Tentative locus assignment
		Cross with SA 291	Cross with SA 222	
Chl^r	13	0[a]	0[a]	D
NR$^-$	4	0[a]	0[a]	
Chl^r	11	0[a]	0.01–0.08[b]	A
NR$^-$	1	0[a]	0.08[b]	
Chl^r	13	0.05–0.11[b]	—	E
NR$^-$	6	0.07–0.13[b]	—	
Chl^r	7	1.00[a]	—	B
NR$^-$	1	0.96[a]	—	
Chl^r	1	0.31[a]	—	C
NR$^-$	15	0.24–0.65[a]	—	

[a] 132 Gal$^+$ colonies were tested for the NR character.
[b] 528 Gal$^+$ colonies were tested for the NR character.

reflects the frequency of crossovers between the end point of the deletion and the point of the mutant allele carried by the Hfr. This class exhibited a range of recombinant frequencies which was clearly distinct from that of the other classes (Table 2).

Since a *chl* B mutant allele would be distal to the selected marker, those mutants which gave 100% Gal$^+$ NR$^+$ recombinants were tentatively assigned to this class (Table 2). All but mutant 216-1 showed contransduction of the *chl* allele with the *met* E marker (Table 3). Strain 216-1 was mated with several multiply marked recipient strains and the mutant allele appeared to be closely associated with *leu* at one minute on the *E. coli* chromosome and therefore distinct from the other five *chl* loci (Table 4A). P1 transduction revealed a 60% cotransduction frequency with *thr* (Table 4B). In order to determine the exact location of the new *chl* mutant locus, a three-point cross was performed using a recipient carrying a *pyr* A marker and the 216-1 *chl* allele. A P1 lysate was prepared from a *thr*$^-$ *pyr* A$^+$ strain which is NR$^+$ and used to transduce the recipient. Pyr$^+$ recombinants were selected and tested for NR$^+$ and for the threonine requirement (Table 5). The least frequent class, Pyr$^+$ Thr$^-$ NR$^-$, represents the double crossover class indicating that the most probable order is *thr-chl-pyr* A. The two distal markers, *pyr* A and *thr* showed a cotransduction frequency of 0.52, *chl* and *pyr* A cotransduce at a frequency of 0.63, but *chl* is more closely linked to *thr* with a cotransduction frequency of 0.78.

Mutants which gave approximately 50% Gal$^+$ NR$^+$ recombinants in the cross with SA 291 were classified as *chl* C (Table 2) since this locus is proximal to the selected marker and unlinked to *gal*. However, when examined for their ability to cotransduce the *chl* allele with *trp*, these *chl* C mutants appeared to be of two types. As seen in Table 6, one group of mutants had an average cotransduction frequency with *trp* B of 0.51, which is similar to a value of 0.46 reported

Table 3. Cotransduction of *chl* B mutants with *met* E

Mutant Strain	Mutant selection procedure	Number of NR⁻ / Total Met⁺ scored	NR⁻ (%)
103-2	Chl^r	19/74	26
108-1	Chl^r	6/42	14
114-2	Chl^r	47/132	36
117-1	Chl^r	6/32	19
118-2	Chl^r	46/132	35
125-2	Chl^r	64/132	48
134-4	Chl^r	3/15	19
216-1	NR⁻	0/132	0

Transduction: Donor PK 27 Met E⁺ NR⁺; Recipient JG 108 Met E⁻ NR⁺.

Table 4. Mapping the *chl* Allele of Strain 216-1

A. Conjugation: Hfr 216-1 NR⁻ Str-s
F⁻ SA 291 Gal⁻ NR⁻ Str-r
AT 3143 Pyr C⁻ Pur E⁻ Pro C⁻ Ilv⁻ Str-r
AB 2102 Pro B⁻ Leu⁻ Str-r

Recipient	Selected marker	No. NR⁻ / Total scored	Linkage of NR⁻ with selected marker (%)
SA 291	Gal⁺	5/132	4
AT 3143	Pyr C⁺	2/88	2
	Pur E⁺	13/132	10
	Pro C⁺	23/87	26
	Ilv⁺	24/89	26
AB 2102	Pro B⁺	65/132	49
	Leu⁺	103/132	78

B. Transduction:
Recipient AB 2102 Pro B⁻ Leu⁻ Thr⁻
Donor 216-1 NR⁻

Selected marker	No. NR⁻ / Total scored	Cotransduction (%)
Pro B⁺	0/56	0
Leu⁺	16/132	10
Thr⁺	104/176	60

by Guest (1969) for cotransduction of *chl* C mutants with *trp* A. Strains 216-2 and 218-1 were less closely linked to tryptophan and seemed to represent a mutant class distinct from the other *chl* C mutants.

Table 5. Three-point cross to determine position of the 216-1 mutant allele

Donor: AB 2102 Thr$^-$ NR$^+$ Pyr A$^+$
Recipient: Thr$^+$ NR$^-$ pyr A$^-$

Selected marker	No. scored	Phenotype			
		Thr$^+$ NR$^+$	Thr$^-$ NR$^+$	Thr$^+$ NR$^-$	Thr$^-$ NR$^-$
Pyr A	220	30	109	76	5

Table 6. Cotransduction of NR$^-$ strains with tryptophan

Donor: PK 27 Trp B$^+$ NR$^-$
Recipient: X997 Trp B$^-$ NR$^+$

Strain	No. NR$^-$/Trp B$^+$ colonies	NR$^-$ (%)
102-1	55/132	42
138	58/132	44
201-1	79/132	60
208-2	68/132	52
210-1	78/132	60
211-1	64/132	49
215-1	70/132	53
217-1	73/132	55
216-2	1/132	<1
218-1	8/88	9

All of the mutants selected by chlorate resistance were unable to reduce nitrate with formate as an electron donor. However, when the mutant strains selected by the latter procedure were tested for chlorate resistance, many were found to be sensitive or partially sensitive to chlorate. Of the 27 strains selected by their inability to reduce nitrate with formate as the electron donor, eight were completely inhibited by 0.5% potassium chlorate and ten grew only very poorly under these conditions. While sensitivity to chlorate could be the result of partial defects which permit some reduction of chlorate, such a correlation is not invariable. For example, mutant 216-1, which mapped near threonine, was chlorate resistant although it possessed 20% of the wild type activity for the formate-nitrate reductase pathway.

Discussion

Table 7 summarizes the mapping results for the chlorate resistant mutants and the NR$^-$ mutants. The frequency of chlorate resistant mutants of PK 27 was approximately 10^{-4} for nitrosoguanidine treated cells while NR$^-$ mutants occurred about fifty times more frequently. The distribution of chlorate resistant mutants selected from mutagenized cells was essentially the same as that reported by Casse (1970) for spontaneous mutants. Ninety-eight per cent of Chl^r mutants fell into the pleiotropic *chl* A, B, D and E classes; only one mutant was recovered

Table 7. Summary of Mutant Selection

Mutant class	Chl^r mutants		NR^- mutants		Total number
	Number	%	Number	%	
chl A	11	24	1	4	12
chl B	7	16	0	0	7
chl C	1	2	13	48	14
chl D	13	29	4	15	17
chl E	13	29	6	22	19
chl F	0	0	2	7	2
chl G	0	0	1	4	1
	45	100	27	100	72

in the *chl* C class. In contrast, of the mutants selected by the NR^- character, 48% were *chl* C mutants. Furthermore two new mutant classes were identified among mutants selected by this procedure.

Strains 216-2 and 218-1, appear to represent a class of mutants distinct from *chl* C strains, showing less than 10% cotransduction with the *trp* B marker. We propose that the designation *chl* F be given this new class of mutants. Guest (1969), in his study of *chl* C mutants reported that five of his strains yielded cotransduction values of 3% or less with a *trp* A marker. These five strains may be analogous to the *chl* F class. The strains 216-2 and 218-1 also appear to be distinct biochemically from *chl* C mutants since they have between 50 and 100% of wild-type activity for the terminal enzyme, nitrate reductase. However, they are defective in the overall activity from formate to nitrate and possess less than 10% of wild-type formate dehydrogenase. It is possible that the *chl* F locus represents the structural gene for formate dehydrogenase and that it is linked to the *chl* C gene coding for nitrate reductase.

The *chl* allele of strain 216-1 failed to map at any of the known *chl* loci but could be placed between *thr* and the *pyr* A marker at 0 minutes on the *E. coli* chromosome. We propose that mutants mapping at this position be designated *chl* G.

The results presented here suggest that chlorate resistance selects only for the most severe alterations in the nitrate reductase pathway. Ninety-eight per cent of the mutants recovered by this procedure are defective in both the formate-nitrate reductase pathway and the formate-hydrogenlyase pathway. Even the *chl* C mutant recovered by this procedure has less than 1% of the wild-type levels of the formate-hydrogenlyase activities. This level of activity, however, is apparently sufficient to allow gas formation in agar stabs. As determined biochemically, all of the other *chl* C mutants also showed varying levels of the formate-hydrogenlyase activity ranging from 5% to 90% of the wild-type activity. Therefore, the *chl* C class of mutants is also pleiotropic although the effect on the formate-hydrogenlyase pathway is less severe. The NR^- mutants not only occur more frequently but they also represent a different distribution of mutant types, including the two new mutant classes. Since many of the NR^- mutants are sensitive or partially sensitive to chlorate, we conclude that selection for chlorate resistance

eliminates most of these mutants and selects for more severe pleiotropic mutations. Although not all the NR^- strains are chlorate resistant we believe it is preferable to maintain a uniform *chl* prefix for all mutants of the nitrate reductase system until the role of each gene is more clearly defined.

The genetics of the nitrate reductase pathway undoubtedly reflects the complexity of the system itself. The components of the pathway must interact in a specific way with one another and with the cell membrane. The system is regulated both by oxygen and the presence of nitrate. Further, it is possible that components of the nitrate reductase system may be components of other systems and it is also possible that there may be alternative electron donors for this pathway depending upon conditions of growth (Lester and DeMoss, 1971). In view of these complexities, it might be expected that mutations in many genes would produce a nitrate reductase negative phenotype. Many of these may be lethal to the cell if they involve components shared by other systems and many mutations may only partially affect formate-nitrate reductase activity if they involve, for example, alternative electron donor systems or if they affect the regulation of the pathway. Such leaky mutants would probably not be recovered among the chlorate resistant strains. For these reasons it is not surprising that new mutant classes have been recovered by selecting strains unable to carry out a formate-dependent nitrate reduction.

References

Adelberg, E., Mandel, M., Chen, G.: Optimal conditions for mutagenesis by N-methyl-N′-nitro-N-nitrosoguanidine in *Escherichia coli* K 12. Biochem. biophys. Res. Commun. **18**, 788–795 (1965).

Adhya, S., Cleary, P., Campbell, S.: A deletion analysis of prophage lambda and adjacent genetic regions. Proc. nat. Acad. Sci. (Wash.) **61**, 956–962 (1968).

Casse, F.: Mapping of the gene *chl*-B controlling membrane bound nitrate reductase and formic hydrogen-lyase activities in *Escherichia coli* K 12. Biochem. biophys. Res. Commun. **39**, 429–436 (1970).

Glaser, J. H., DeMoss, J. A.: Phenotypic restoration by molybdate of nitrate reductase activity in *chl* D mutants of *Escherichia coli*. J. Bact. **108**, 854–860 (1971).

Gray, C. T., Gest, H.: Biological formation of molecular hydrogen. Science **148**, 186–192 (1965).

Guest, J. R.: Biochemical and genetic studies with nitrate reductase C-gene mutants of *Escherichia coli*. Molec. gen. Genetics **105**, 285–297 (1969).

Iida, D., Taniguchi, S.: Studies on nitrate reduction of *Escherichia coli*. I. Particulate electron transport system to nitrate and its solubilization. J. Biochem. (Tokyo) **46**, 1041–1055 (1959).

Kahn, P. L.: Isolation of high-frequency recombining strains from *Escherichia coli* containing the V colicinogenic factor. J. Bact. **96**, 205–214 (1968).

Lennox, E. S.: Transduction of linked genetic characters of the host by bacteriophage Pl. Virology **1**, 190–206 (1955).

Lester, R. L., DeMoss, J. A.: Effects of molybdate and selenite on formate and nitrate metabolism in *Escherichia coli*. J. Bact. **105**, 1006–1014 (1971).

Peck, H. D., Jr., Gest, H.: Formic dehydrogenase and the hydrogenlyase enzyme complex in coli-aerogenes bacteria. J. Bact. **73**, 706–721 (1957).

Piechaud, M., Puig, J., Pichinoty, F., Azoulay, E., LeMinor, L.: Mutations affectant la nitrate-reductase et d'autres enzymes bacteriennes d'oxydo-reduction. Ann. Inst. Pasteur **112**, 24–37 (1967).

Puig, J., Azoulay, E., Pichinoty, F.: Etude génétique d'une mutation à effet pleiotrope chez l'*Escherichia coli* K_{12}. C. R. Acad. Sci. (Paris) **264**, 1507–1509 (1967).

Ruiz-Herrera, J., DeMoss, J. A.: Nitrate reductase complex of *Escherichia coli* K-12: Participation of specific formate dehydrogenase and cytochrome b_1 components in nitrate reduction. J. Bact. **99**, 720–729 (1969).

Ruiz-Herrera, J., Showe, M. K., DeMoss, J. A.: Nitrate reductase complex of *Escherichia coli* K-12: Isolation and characterization of mutants unable to reduce nitrate. J. Bact. **97**, 1291–1297 (1969).

Taylor, A. L.: Current linkage map of *Escherichia coli*. Bact. Rev. **34**, 155–175 (1970).

Venables, W. A., Guest, J. R.: Transduction of nitrate reductase loci of *Escherichia coli* by phages P1 and λ. Molec. gen. Genetics **103**, 127–140 (1968).

Wimpenny, J. W. T., Cole, J. A.: The regulation of metabolism in faculative bacteria. III. The effect of nitrate. Biochim. biophys. Acta (Amst.) **148**, 233–242 (1967).

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