JOURNAL OF BACTERIOLOGY, Nov. 1996, p. 6348–6351 Vol. 178, No. 21
0021-9193/96/$04.00+0

NOTES

The Temperature-Sensitive Growth and Survival Phenotypes of *Escherichia coli cydDC* and *cydAB* Strains Are due to Deficiencies in Cytochrome *bd* and Are Corrected by Exogenous Catalase and Reducing Agents

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Received 8 April 1996/Accepted 14 August 1996

The *cydDC* operon of *Escherichia coli* encodes an ATP-dependent transporter of unknown function that is required for cytochrome *bd* synthesis. Strains containing defects in either the *cydD* or *cydC* gene also demonstrate hypersensitivity to growth at high temperatures and the inability to exit the stationary phase at 37°C. We wished to determine what is responsible for these hypersensitive phenotypes and whether they are due to a lack of the CydDC proteins or a defect of the cytochrome *bd* encoded by the *cydAB* genes. Using both K-12- and B-type strains of *E. coli*, we have compared the phenotypes of isogenic *cydAB* mutants and *cydC* mutants. In both K-12- and B-type backgrounds, the hypersensitive phenotypes are due to defects of cytochrome *bd* activity and not defects of the *cydDC* genes. We also found that the temperature-sensitive growth phenotypes can be suppressed by exogenous reducing agents, such as glutathione and cysteine. Strikingly, even the enzymes catalase and superoxide dismutase, when added exogenously, can correct the temperature-sensitive and stationary phase arrest phenotypes. We propose that the temperature sensitive growth phenotypes are due to a buildup of diffusible oxygen radicals brought on by the absence of cytochrome *bd*.

There are two major terminal oxidases in the cytoplasmic membrane of *Escherichia coli* when cells are grown under aerobic conditions: cytochrome *bo*, encoded by the *cyoABCDE* operon, and cytochrome *bd*, encoded by the *cydAB* operon (1). Both complexes catalyze the oxidation of ubiquinol and reduce oxygen to water with the generation of an electrochemical proton gradient across the membrane. When cells are grown under conditions of high oxygen tension, cytochrome *bo* predominates. Under conditions of lower oxygen tension, such as during late-exponential-phase and stationary-phase growth, cytochrome *bd* activity is induced (1). Transcription of the *cydAB* genes also appears to be induced by heat shock, an effect mediated by the ArcAB proteins (17).

In *E. coli*, the *cydDC* genes form an operon and are predicted to encode a heterodimeric ATP-binding cassette type transporter (13) that is required for the synthesis of cytochrome *bd* and all *c*-type cytochromes. The substrate for this putative transporter is unknown. Strains containing defects in either the *cydD* or *cydC* gene have been isolated in a number of laboratories by a variety of genetic screens. These defects include the loss of cytochrome *bd* activity (*cyd*) (6, 14), the inability to grow at 42°C (*htrD*) (4), and the inability to exit stationary phase when grown at 37°C (*sur*) (15). In addition, *cydD* mutants were shown to exhibit a high reversion rate (as high as 10^{-3}); an increased sensitivity to hydrogen peroxide (H_2O_2), zinc, and aniline dyes; and an increased uptake of cysteine (4, 5, 12, 15, 17).

Although some of these phenotypes have also been reported for strains containing defects in the *cydAB* genes (5, 15, 17), a comprehensive study with *E. coli* K-12 and B strains has not been carried out to establish which of the *cydDC* phenotypes are attributed to a deficiency of the cytochrome *bd* or the CydDC transporter itself. Understanding the phenotypes that are specifically due to defects in the *cydDC* genes and not cytochrome *bd* is essential to the eventual determination of the substrate of the CydDC transporter.

In this study, we have analyzed phenotypes associated with a *cydC* mutant and compared them with the phenotypes associated with an isogenic *cydAB* mutant. Our results demonstrate that the temperature-sensitive and stationary-phase survival defects associated with *cydDC* mutants are attributable to a deficiency of cytochrome *bd*. We find that these defects can be corrected by external reducing agents or by the enzymes catalase and superoxide dismutase. These results demonstrate that the cytochrome *bd* is directly or indirectly necessary to protect the cell from oxidative damage, a conclusion that is consistent with the ArcAB-mediated induction patterns and some properties of *cydAB* strains previously reported (4, 5, 9, 15, 17).

Comparison of *cydC* and *cydAB* mutant phenotypes. Reports in the literature have been inconsistent with regard to the phenotypes associated with *cydDC* and *cydAB* mutants. For example, the first reports of isolating *cydC* and *cydD* mutants do not mention that these strains are temperature sensitive (6, 14), yet *cydD* mutants were later isolated as temperature sensitive (4). In addition, the report on the stationary-phase arrest phenotype suggests that *cydD* (*surB*) mutants are more affected than the *cydAB* mutants (15). Future studies will require an understanding of whether phenotypic differences between *cydAB* and *cydDC* strains are due to the parental strains used or to the high reversion rate of *cydDC* mutants (17) or to actual biochemical differences.

To elucidate the differences between *cydDC* and *cydAB* mu-

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype	Source or reference
E. coli K-12		
GO103	Δ*cydAB*::Kn^r	11
GN04	*cydC*::λMu*d*Kan	6
TB1	*hsdR ara* Δ(*lac-proAB*) *rpsL* [Φ80d *lac*(Δ*lacZ*)*M15*]	2
RGK264	*cydC*::λMudKan in TB1	This work
RGK265	Δ*cydAB*::Kn^r in TB1	This work
E. coli B		
BL21	λDE3 T7 gene *1* (RNA polymerase) *ompT hsdS*	Novagen
RGK270	*cydC*::λMudKan in BL21	This work
RGK271	Δ*cydAB*::Kn^r in BL21	This work
Plasmid pTK1	Ap^r; *cydAB*	R. B. Gennis

tants, we transduced mutant alleles of both *cydAB* (Δ*cydAB*::Kan) and *cydC* (*cydC*::λMud*lac*) into the *E. coli* K-12-type strain TB1 and B-type strain BL21(DE3) by using phage P1 (Table 1) and compared their phenotypes. In these backgrounds, both mutations result in a similarly high reversion rate. To ensure that the experimental results derived from the present studies were not due to reversion, all lysates were prepared and transductions were performed at 30°C. The results indicate that all of the *cyd* phenotypes shown in Table 2 are due to a deficiency of cytochrome *bd*, whether the background was *E. coli* B or K-12 strains.

Overexpression of the *cydAB* genes corrects most CydC defects. Upon transformation of plasmid pTK1, containing the *cydAB* genes, into a *cydC* mutant, we noticed that the temperature-sensitive defect of the *cydC* strain was corrected. We wanted to determine if the transformants made cytochrome *bd* and if other defects might be corrected. Plasmid pTK1 was transformed into the *cydC E. coli* K-12 (TB1) and B (BL21) mutants. Membrane fractions from B-type *E. coli* cells grown overnight in Luria broth (LB) medium containing 0.2% glycerol were solubilized in Triton X-100 and analyzed spectrophotometrically (Fig. 1). Cytochrome *bd* is characterized, in reduced-minus-oxidized spectra, by three peaks derived from the two *b*-type hemes (b_{558} and b_{595}) and one *d*-type heme (approximately 630 nm). The *cydC* mutant makes no detectable cytochrome *bd*, while the wild type exhibits the diagnostic peaks. However, the *cydC* mutant containing the pTK1 plasmid makes significant levels of cytochrome *bd*, although less than the wild-type strain containing pTK1.

Although other explanations are possible, it is likely that this *cydC* defect is overcome by overproducing the cytochrome *bd*

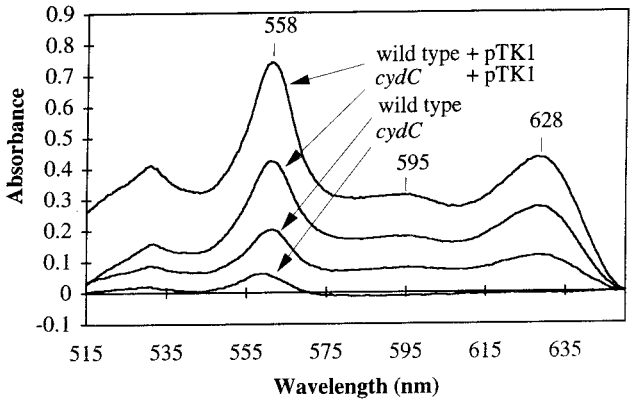

FIG. 1. Difference spectroscopy of membrane fractions showing reduced-minus-oxidized spectra of the Triton X-100-solubilized membrane fraction of *cydC* and wild-type (BL21) strains with and without the plasmid pTK1 containing the *cydAB* genes. Protein concentrations were approximately 11 mg/ml in all strains.

TABLE 2. Phenotypes of *cydAB*, *cydC*, and wild-type strains[a]

Strain (genotype)	Phenotype						
	Growth in the presence of[b]:					Increased stationary-phase arrest[c]	Cytochrome *bd*[d]
	LB at 30°C	LB at 37°C	LB at 42°C	H_2O_2	Zinc		
TB1 (K-12 wild type)	+	+	+	R	R	No	+
RGK264 (*cydC*)	+	±	−	S	S	No	−
RGK265 (*cydAB*)	+	±	−	S	S	No	−
BL21 (B wild type)	+	+	+	R	R	No	+
RGK270 (*cydC*)	+	±	−	S	S	Yes	−
RGK271 (*cydAB*)	+	±	−	S	S	Yes	−

[a] The data represent our results of experiments that were originally performed with *E. coli* K-12 derivative strains in the papers described below. References 6 and 14 first demonstrated the defects for cytochrome *bd* in *cydC* and *cydD* mutants, respectively. The high-temperature sensitivity phenotype for the *cydD* (*htrD*) and *cydAB* mutants was first described in references 4 and 15, respectively. Hydrogen peroxide sensitivity was first demonstrated for both *cydD* and *cydAB* mutants in reference 17. Zinc sensitivity was first demonstrated in *cydD* mutants in reference 14 and in *cydD* (*htrD*) and *cydAB* mutants in reference 5. Seigele and Kolter (15) demonstrated the stationary-phase defect in *cydD* (*surB*) and, to a lesser extent, *cydAB* mutants.

[b] +, good growth; ±, poor growth; −, no growth (as determined by growth of single-colony isolates on LB plates after 24 h). R, resistant; S, sensitive (as determined by the presence or absence of a zone of growth inhibition around a sterile disk containing either 5 μl of 30% H_2O_2 or 0.4 M $ZnCl_2$ placed on 0.1 ml of cells grown to saturation at 30°C and plated onto LB medium).

[c] Stationary-phase arrest on solid medium (see the text); all *cyd* mutants in both *E. coli* backgrounds arrest after 8 to 12 h in liquid medium (described in reference 13).

[d] Cytochrome *bd* was determined by reduced-minus-oxidized spectral analysis of Triton X-100-solubilized membrane fractions.

TABLE 3. Suppression of the stationary-phase arrest and high-temperature sensitivity phenotypes of *cydAB* and *cydDC* mutants[a]

Correct stationary-phase arrest and high-temperature phenotypes	Do not correct stationary-phase arrest and high-temperature phenotypes
Reduced glutathione	Oxidized glutathione
L-Cysteine	Cystine
D-Cysteine	Sulfate
DTT + oxidized glutathione	Methionine
DTT + cystine	Nitrate
Ascorbic acid	Heme
MESNA	Glutamine
NADH	Alanine
NADPH	
Sodium hydrosulfite	
Superoxide dismutase	
Catalase	

[a] Cultures of *cydC* and *cydAB* cells were grown to stationary phase in LB medium at 30°C and diluted 10^{-3}. Cells (0.1 ml) were added to 3 ml of LB medium containing 0.6% agar at 58°C. This mixture was overlaid onto solid LB medium, sterile disks were placed on these plates, and various reducing and nonreducing agents were added. These cells were grown overnight at 30 and 42°C. Reagents were scored positive for phenotype correction if colonies formed around the disks at both temperatures.

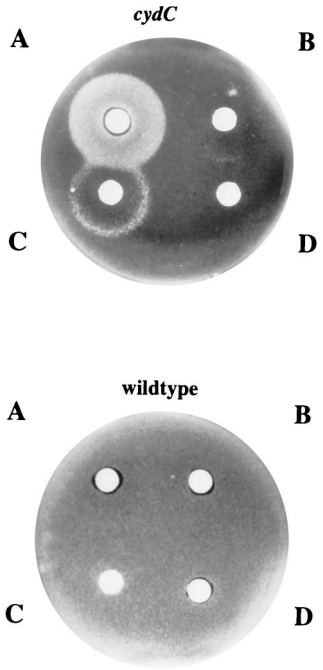

FIG. 2. Suppression of stationary-phase arrest in *cyd* mutants by exogenous reducing agents. Cells grown to stationary phase were diluted 10^{-3}, and 0.1 ml was inoculated into 3 ml of LB liquid medium containing 0.6% agar. These cells were overlaid onto LB medium, and disks were placed onto these plates. Solutions containing reducing agents were added to the disks, and the cells were grown at 30°C. Disks: A, reduced glutathione (5 μl of 1 M solution); B, DTT (5 μl of 1 M solution); C, both oxidized glutathione and DTT (5 μl each of 1 M solutions); D, oxidized glutathione (5 μl of 0.5 M solution).

polypeptides, with a smaller percentage forming the active cytochrome. This result allowed us to confirm which phenotypes are due to a *cydDC* transporter defect and which are due to a cytochrome *bd* deficiency. Phenotypically, the *cydC*/pTK1 strain grew as well as the wild type at 42°C, did not arrest at stationary phase when grown at 37°C, and was not hypersensitive to zinc or hydrogen peroxide. These results were observed for both the K-12- and the B-type *E. coli* backgrounds. To ensure that the suppression of the *cydC* phenotype by the plasmid was not due to reversion, we transformed the pTK1 plasmid into *cydC* mutants in both the K-12- and B-type strains and screened 50 different colonies for the temperature- and zinc-sensitive phenotypes. For both *E. coli* strains, 50 of 50 transformant colonies grew at 42°C and were zinc insensitive. We conclude that the properties of the *cydC* mutant strains, including the stationary-phase defect, are due to a deficiency of cytochrome *bd* and not to an intrinsic defect of the *cydDC* transporter.

The high-temperature sensitivity and stationary-phase arrest phenotypes are corrected by exogenous reducing agents and catalase. Siegele and Kolter (15) isolated and described *cydD* mutants (*surB*) in which cell division arrests at stationary phase when grown at 37°C but not at 30°C. Both our *E. coli* B and K-12 *cydC* and *cydAB* cells, when taken from a stationary-phase culture and reinoculated into fresh LB broth, arrested at stationary phase for 8 to 12 hours at 37°C and then continued to grow. We were able to increase this time of arrest on solid medium up to 2 days in the *E. coli* B-type strain by heat shocking the cells for a few seconds; we were unable to increase stationary-phase arrest in the K-12 background. Using the B-type strain, we developed a plate assay to test compounds that might correct the high-temperature defects. We grew *cydC*, *cydAB*, and wild-type cells (of the BL21 background) at 30°C to stationary phase. These cultures were diluted 10^{-3}, and 0.1 ml was added to 3 ml of LB medium containing 0.6% Bacto-Agar at 58°C. These cells were then overlaid onto LB medium and incubated overnight at 30°C. No growth was seen on the plates containing the *cydAB* and *cydC* mutants after 24 h, but a lawn of cells was observed after 48 to 72 h. On plates containing wild-type cells, a lawn was observed after 24 h.

While analyzing an array of compounds, we observed that low-molecular-weight reducing agents could correct the arrest phenotype of *cydC* mutants (Fig. 2). Therefore, we tested these reducing compounds on both *cydAB* and *cydC* mutants and analyzed the arrest phenotype. A small disk of sterile filter paper (3 MM; Whatman, Fairfield, N.J.) was placed on the plate containing heat shocked cells. Various reducing agents, made at 1.0 or 0.5 M concentrations, were then added in 5-μl portions to the disks. The plates were incubated at 30°C, and growth of the mutant around the disk was analyzed after 24 h. We also tested the correction of the temperature-sensitive growth phenotype of the *cyd* mutants by growing cells at 42°C that had been treated in the same manner (Table 3).

The thiol reducing agents glutathione, L-cysteine, and D-cysteine suppressed both the survival and temperature-sensitive phenotypes. There was a zone of inhibition around the disks containing cysteine, possibly as a result of the low pH of dissolved cysteine (Fig. 2). Non-thiol reducing agents, such as ascorbic acid, NADH, and NADPH, also suppressed the phenotypes. The reducing agent dithiothreitol (DTT) only partially corrected the phenotypes, since a thin halo of tiny colonies grew a few millimeters from the disk of DTT. Oxidizing agents such as cystine, oxidized glutathione and sulfate, and amino acids such as methionine, alanine, and glutamine did not suppress the survival and temperature-sensitive phenotypes. When DTT was combined with oxidized glutathione or cystine,

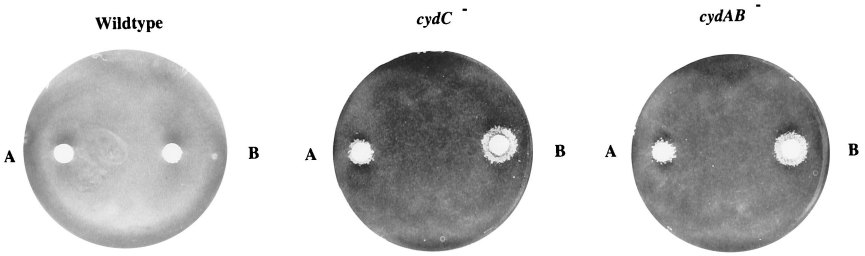

FIG. 3. Temperature-sensitive growth of *cyd* mutants is corrected by exogenous catalase and superoxide dismutase. Cells grown to stationary phase were diluted 10^{-3}, and 0.1 ml was inoculated into 3 ml of LB liquid medium containing 0.6% agar. These cells were overlaid onto LB medium, and disks were placed onto these plates. Solutions containing enzymes were added to the disks, and the cells were grown at 42°C. Disks: A, bovine superoxide dismutase (5 μl of a 20-mg/ml solution); B, bovine catalase (5 μl of a 100-mg/ml solution).

the phenotypes were again suppressed as large halos around the disks. The reducing agent β-mercaptoethanesulfonic acid (MESNA) also suppressed both the stationary-phase and temperature-sensitive phenotypes (Table 3). Since MESNA is believed to be membrane impermeable, this result suggests that the active agent(s) functions extracellularly.

The ability of extracellular reducing agents to correct the temperature-sensitive phenotypes of *cyd* mutants suggests that specific elements in the periplasm must be at least partially responsible for these defects. A deficiency of a cytochrome could lead to more reduced intermediate electron carriers and subsequently to the production of oxygen radicals and H_2O_2 after reaction of oxygen with them (16). We tested this by determining the effect of exogenous catalase and superoxide dismutase on the temperature sensitive and arrest phenotypes. These enzymes are naturally involved in protecting the cell from damage by oxygen radicals. We added 5 μl of a 100-mg/ml filter-sterilized solution of bovine liver catalase or a 20-mg/ml solution of bovine liver superoxide dismutase (Sigma, St. Louis, Mo.) to disks of sterile filter paper. These were placed onto heat-shock treated cells overlaid in 0.6% agar on LB plates. After growth at 30 and 42°C (Fig. 3), it was observed that both enzymes corrected the high-temperature and stationary phase phenotypes. Superoxide dismutase converts superoxide anion to hydrogen peroxide and molecular oxygen; catalase disproportionates hydrogen peroxide to water and oxygen.

On the basis of these results, we propose that a deficiency of cytochrome *bd* results in an increase in both superoxide anion and H_2O_2 levels in the periplasm. Under some conditions, these oxidative agents may also freely diffuse to adjacent cells, causing the stationary-phase arrest and temperature-sensitive phenotypes. It is also likely that *cyd* mutants cannot naturally destroy these agents as well as the wild type does, a hypothesis consistent with the increased sensitivity of *cyd* mutants to H_2O_2. The reason for the inability to remove oxidative agents is unclear. The exogenously added catalase could act as a "sink" for periplasmically generated hydrogen peroxide, thereby creating increased diffusion outward from the cell. A very recent paper (10) has found that both *cydAB* and *cydD* mutants of *E. coli* and *cydD* mutants of the pathogen *Providencia stuartii* have growth-sensitive phenotypes in response to a self-produced diffusible factor. Our results suggest that this factor could be H_2O_2 or a similar oxygen radical species. Thus, cytochrome *bd*-dependent stationary-phase arrest and temperature-sensitive growth can be corrected by external reducing agents and oxygen radical scavenging enzymes. Recently, Imlay and Imlay have cloned the *E. coli sodC* gene encoding a periplasmic CuZn superoxide dismutase (8). Clearly, the periplasmic as well as the cytoplasmic compartments of gram-negative bacteria must be protected from oxidative damage. Nevertheless, the exact target(s) of the oxidative agents (3) responsible for the stationary-phase arrest and temperature-sensitive growth remains to be determined.

Conclusions. Because of the similarity of the reported phenotypes of *cydAB* and *cydDC* mutants, we thought that it was necessary to determine which properties of *cydDC* mutants are due to a deficiency of cytochrome *bd* in different *E. coli* backgrounds. We conclude that hypersensitivity to high temperature, H_2O_2, zinc, and stationary-phase arrest can be attributed solely to a deficiency of the cytochrome *bd* in a *cydC* mutant. Stationary-phase arrest and high-temperature sensitivity of *cydC* and *cydAB* mutants are corrected by reducing agents and the exogenously supplied enzymes catalase and superoxide dismutase. It is likely that a critical component, possibly within the periplasm, must be reduced (or prevented from oxidation) to prevent this stationary-phase arrest and temperature sensitivity. Thus, *cyd* mutants may be overproducing H_2O_2 (or other oxygen radicals) and/or unable to remove them. This is in keeping with the result that there is an increase in metal-catalyzed oxidation by electron transport components when there is a deficiency of a terminal electron acceptor (16). We conclude that cytochrome *bd* activity is necessary for protecting the cell from oxidative damage when cells are grown aerobically during stationary phase and at high temperatures. This conclusion is consistent with the transcriptional induction pattern of the *cydAB* genes, in which the oxidative-sensing system ArcAB controls activation of the operon (9). Thus, in addition to providing a proton motive force for cells faced with low oxygen tensions, this cytochrome may reduce the levels of oxidative damage, directly or indirectly, during these times. An accompanying paper describes some differences between *cydAB*, *cydDC*, and wild-type strains with respect to the ability to reduce periplasmic disulfide-containing proteins or to synthesize heme proteins (7).

We thank J. Ghaim and R. Gennis for strains and plasmids and B. Bowman, P. Cullen, and B. Kunkel for comments on the manuscript.

This work was supported by NIH grant GM47909 to R.G.K.

REFERENCES

1. **Anraku, Y., and R. B. Gennis.** 1987. The aerobic respiratory chain of *Escherichia coli*. Trends Biochem. Sci. **12:**262–266.
2. **Baldwin, T.** 1984. *E. coli* TB1-host for pUC plasmids. Focus **6:**7.
3. **Davies, K. J. A., M. E. Delsignore, and S. W. Lin.** 1987. Protein damage and degradation by oxygen radicals. J. Biol. Chem. **262:**9902–9907.
4. **Delaney, J. M., D. Ang, and C. Georgopoulos.** 1992. Isolation and characterization of the *Escherichia coli htrD* gene, whose product is required for growth at high temperatures. J. Bacteriol. **174:**1240–1247.
5. **Delaney, J. M., D. Wall, and C. Georgopoulos.** 1993. Molecular characterization of the *Escherichia coli htrD* gene: cloning, sequence, regulation, and involvement with cytochrome *d* oxidase. J. Bacteriol. **175:**166–175.
6. **Georgiou, C. D., H. Fang, and R. B. Gennis.** 1987. Identification of the *cydC* locus required for expression of the functional form of the cytochrome *d* terminal oxidase complex in *Escherichia coli*. J. Bacteriol. **169:**2107–2112.
7. **Goldman, B. S., K. K. Gabbert, and R. G. Kranz.** 1996. Use of heme reporters for studies of cytochrome biosynthesis and heme transport. J. Bacteriol. **178:**6338–6347.
8. **Imlay, K. R. C., and J. A. Imlay.** 1996. Cloning and analysis of *sodC*, encoding the copper-zinc superoxide dismutase of *Escherichia coli*. J. Bacteriol. **178:**2564–2571.
9. **Iuchi, S., V. Chepuri, H. A. Fu, R. B. Gennis, and E. C. Lin.** 1990. Requirement for terminal cytochromes in generation of the aerobic signal for the *arc* regulatory system in *Escherichia coli*: study utilizing deletions and *lac* fusions of *cyo* and *cyd*. J. Bacteriol. **172:**6020–6025.
10. **Macinga, D. R., and P. N. Rather.** 1996. *aarD*, a *Providencia stuartii* homologue of *cydD*: role in 2′-*N*-acetyltransferase expression, cell morphology and growth in the presence of an extracellular factor. Mol. Microbiol. **19:**511–520.
11. **Oden, K. L., and R. B. Gennis.** 1991. Isolation and characterization of a new class of cytochrome *d* terminal oxidase mutants of *Escherichia coli*. J. Bacteriol. **173:**6174–6183.
12. **Poole, R. K., F. Gibson, and G. Wu.** 1994. The *cydD* gene product, component of a heterodimeric ABC transporter, is required for assembly of periplasmic cytochrome *c* and of cytochrome *bd* in *Escherichia coli*. FEMS Microbiol. Lett. **117:**217–223.
13. **Poole, R. K., L. Hatch, M. W. J. Cleeter, F. Gibson, G. B. Cox, and G. Wu.** 1993. Cytochrome *bd* biosynthesis in *Escherichia coli*: the sequences of the *cydC* and *cydD* genes suggest that they encode the components of an ABC membrane transporter. Mol. Microbiol. **10:**421–430.
14. **Poole, R. K., H. D. Williams, J. A. Downie, and F. Gibson.** 1989. Mutations affecting the cytochrome *d*-containing oxidase complex of *Escherichia coli* K12: identification and mapping of a fourth locus, *cydD*. J. Gen. Microbiol. **135:**1865–1874.
15. **Siegele, D. A., and R. Kolter.** 1993. Isolation and characterization of an *Escherichia coli* mutant defective in resuming growth after starvation. Genes Dev. **7:**2629–2640.
16. **Stadtman, E. R.** 1992. Protein oxidation and aging. Science **257:**1220–1224.
17. **Wall, D., J. M. Delaney, O. Fayet, B. Lipinska, T. Yamamoto, and C. Georgopoulos.** 1992. *arc*-dependent thermal regulation and extragenic suppression of the *Escherichia coli* cytochrome *d* operon. J. Bacteriol. **174:**6554–6562.