Vol. 42, No. 4

Mutations in *aarE*, the *ubiA* Homolog of *Providencia stuartii*, Result in High-Level Aminoglycoside Resistance and Reduced Expression of the Chromosomal Aminoglycoside 2′-*N*-Acetyltransferase

MICHAEL R. PARADISE,[1] GREGORY COOK,[2] ROBERT K. POOLE,[2] AND PHILIP N. RATHER[1,3]*

Department of Medicine and Department of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine,[1] and Research Service, Veterans Affairs Medical Center,[3] Cleveland, Ohio 44106, and Microbial Physiology Research Group, King's College London, London W8 & AH, United Kingdom[2]

The *aarE1* allele was identified on the basis of the resulting phenotype of increased aminoglycoside resistance. The *aarE1* mutation also resulted in a small-colony phenotype and decreased levels of *aac(2′)-Ia* mRNA. The deduced AarE gene product displayed 61% amino acid identity to the *Escherichia coli* UbiA protein, an octaprenyltransferase required for the second step of ubiquinone biosynthesis. Complementation experiments in both *Providencia stuartii* and *E. coli* demonstrated that *aarE* and *ubiA* are functionally equivalent.

The regulation of a 2′-*N*-acetyltransferase [encoded by the *aac(2′)-Ia* gene] involved in peptidoglycan acetylation in the gram-negative enteric bacterium *Providencia stuartii* (8, 9, 29) has been extensively studied in our laboratory. This housekeeping gene was originally identified as an aminoglycoside resistance gene because of the acetylation of certain aminoglycosides by the AAC(2′)-Ia protein (7, 34, 39). A number of regulatory genes that influence expression of *aac(2′)-Ia* have been identified, including *aarA*, *aarB*, *aarC*, *aarD*, and *aarP* (21, 22, 33–35). The *aarD* gene in *P. stuartii* encodes CydD (22), a heterodimeric component of an ABC transporter required for the formation of a functional cytochrome *bd* oxidase (30, 31). This oxidase is required for the terminal step in electron transport under aerobic conditions. A null allele of *aarD* increased *aac(2′)-Ia* transcription (22), suggesting that the regulation of *aac(2′)-Ia* expression is coupled to some aspect of electron transport.

The process of electron transport in bacteria requires the molecule ubiquinone, which is capable of accepting electrons from a variety of dehydrogenases and transferring them to a terminal oxidase. At least nine genes (*ubiAH* and *ubiX*) that are required for ubiquinone biosynthesis have been identified (11, 12, 18, 19, 24, 28, 36–38, 40, 41). The primary role of ubiquinone is in electron transport; however, it has been reported that ubiquinone mutants have reduced numbers of flagella and are nonmotile (15). This phenotype may be related to defects in electron transport.

In this study, we found that a recessive mutation in the *aarE* gene, a homolog of *ubiA*, severely decreases *aac(2′)-Ia* mRNA accumulation. The product of *ubiA* is parahydroxybenzoate octaprenyltransferase, an enzyme involved in the second step of ubiquinone biosynthesis (38, 41). Based on the data obtained in this study, we propose a model in which ubiquinone or a functional electron transport chain is required for a process that influences *aac(2′)-Ia* mRNA accumulation. Our data

suggest that this process does not involve the previously identified transcriptional activator AarP (21).

Bacterial strains and plasmids. *Escherichia coli* XL1 Blue (Stratagene) and DH5α (Gibco/BRL) were used as hosts for transformations. *E. coli* DH5α λ *pir* (21) and SM10 λ *pir* (26) were used as hosts for pKNG101 (16) derivatives. *P. stuartii* PR50 is a wild-type strain and has been described previously (34). Plasmids pACYC184 (6) and pBluescript II SK(−) (Stratagene) were used as cloning vectors. Electroporations were done as described previously (34).

Northern blot analysis. Total RNA was prepared by using the TRizol reagent (Gibco/BRL), and equal amounts were loaded, as determined on the basis of the intensities of the 23S and 16S rRNAs. The RNA was fractionated on a 1.2% agarose gel containing 2.2 M formaldehyde and transferred to nylon membranes by capillary transfer. To ensure that equal amounts of RNA were transferred to the nylon membranes, the nylon filters were photographed while under UV illumination. The filters were then probed with a digoxigenin-labeled 602-bp *Taq*I-*Ssp*I fragment containing the *aac(2′)-Ia* coding sequence. The filters were developed with the LumiPhos substrate.

DNA sequencing. Double-stranded templates were sequenced by using an AutoRead sequencing kit (Pharmacia) with fluorescein-labelled universal and reverse primers. Sequencing reactions were run on an A.L.F. automated sequencer (Pharmacia).

Isolation of the *aarE1* allele. Previous studies have shown that when *P. stuartii* is selected for gentamicin resistance at a level fourfold above the MIC, the resulting mutants usually display enhanced expression of the *aac(2′)-Ia* gene (22, 34, 35). These previous selections were done in cells that contained an *aac(2′)-Ia* fusion on the multicopy plasmid pR401 (33). To avoid the possibility that the presence of the *aac(2′)-Ia* promoter in multiple copies was altering the isolation of *aac(2′)-Ia* regulatory mutants, we reisolated gentamicin-resistant mutants of PR50 lacking pR401. A mutant displaying high-level gentamicin resistance was isolated by plating PR50 (34) on Luria-Bertani plates containing 15 μg of gentamicin per ml. This mutant, PR11, was resistant to gentamicin at 256 μg/ml, relative to the isogenic parent, PR50, which was resistant to gentamicin at 4 μg/ml, and this mutant displayed a small-colony

* Corresponding author. Mailing address: Infectious Diseases Section 1110W, Wade Park Medical Center, 10701 E. Boulevard, Cleveland, OH 44106. Phone: (216) 368-0744. Fax: (216) 368-2034. E-mail: pxr17@po.cwru.edu.

FIG. 1. The *aarE1* allele decreases *aac(2′)-Ia* expression. RNA (20 μg) prepared from PR50 (wild type) and PR11 (*aarE1*) was electrophoresed on a 1.2% formaldehyde gel, transferred to a nylon membrane, and probed with a digoxigenin-labeled *aac(2′)-Ia* probe. The amounts of RNA loaded were standardized to the 23S and 16S rRNAs as an internal control. Both visible bands correspond to *aac(2′)-Ia* message; the upper, less prominent band is occasionally seen and probably represents trapping of the *aac(2′)-Ia* mRNA by rRNA.

phenotype. The mutation responsible for this phenotype was designated *aarE1*, and the increased gentamicin resistance suggested that *aarE1* may increase expression of the *aac(2′)-Ia* gene. To examine this possibility, Northern blot analysis was used to determine the accumulation of *aac(2′)-Ia* mRNA in both the mutant and parental strains at an optical density at 600 nm of 0.6. The results of this analysis are shown in Fig. 1; the levels of *aac(2′)-Ia* mRNA were significantly lower in PR11 *aarE1* than in the wild-type strain PR50. The decreased accumulation of *aac(2′)-Ia* mRNA in PR11 *aarE1* indicated that a mechanism independent of *aac(2′)-Ia* must account for the high-level gentamicin resistance observed for this mutant.

Isolation and characterization of *aarE*. Strain PR11 exhibited a reduced growth rate and formed significantly smaller colonies than PR50 on Luria-Bertani agar plates. In addition, PR11 exhibited a reduced growth yield in liquid culture, with growth ceasing at an optical density at 600 nm of 0.4 to 0.5. Since PR11 was a spontaneous mutant, it seemed likely that a single mutation was responsible for both the reduced growth rate and the regulatory effects on *aac(2′)-Ia*. Based on this assumption, a library of PR50 genomic DNA was constructed in pACYC184 (6) and introduced into PR11. Transformants with a normal growth rate were easily visible in the background of microcolonies. Plasmid DNA was purified from several large colonies and retransformed into PR11, with 100% of the transformants exhibiting a wild-type growth rate. Analysis of a complementing plasmid, pAFM11, indicated the presence of a 2.1-kb insert. The 2.1-kb *Sal*I-*Eco*RV fragment was cloned into pBluescript II SK(−), resulting in plasmid pSK.aarE, which was also capable of complementing the *aarE1* allele. The nucleotide sequence was determined from each end of the insert. At one end of the insert in pSK.aarE, a partial open reading frame encoding 79 amino acids with 76% identity to amino acids 710 to 789 of PlsB, which is the *E. coli* glycerol-3-phosphate *O*-acyltransferase, was identified (20). Nucleotide sequence analysis of the other end of the insert revealed two

open reading frames. The first open reading frame, proximal to the end of the insert, encoded a truncated protein with 65% identity to the carboxy-terminal 48 amino acids of the *E. coli* UbiC protein (28). The second open reading frame, immediately adjacent to the first open reading frame, encoded a predicted product with 67% amino acid identity to amino acids 1 to 103 of the *E. coli* UbiA protein, which is *p*-hydroxybenzoate octaprenyltransferase (38, 41). Thus, the organization of this region of the *P. stuartii* chromosome is identical to that of *E. coli*, and it was predicted that the only functional gene contained within pSK.aarE would be the *ubiA* homolog. This indicated that the *aarE1* mutation was within this *ubiA* homolog. On the basis of this information, the remainder of the *ubiA* sequence was determined, revealing a single complete open reading frame of 864 nucleotides capable of encoding a product of 288 amino acids. The product of this open reading frame, designated AarE, displayed 61% amino acid identity and 74% similarity to the *E. coli* UbiA protein, an octaprenyltransferase required for the second step of ubiquinone biosynthesis.

The *aarE* and *ubiA* genes are functionally equivalent. To determine whether PR11 was deficient in the production of ubiquinone, two tests were performed. First, growth on medium containing succinate as the sole carbon source requires ubiquinone under aerobic conditions (11). PR11 was unable to grow under these conditions, whereas the wild-type strain PR50 grew well. Next, cell extracts of PR11 (*aarE1*) and PR50 (wild type) were directly examined for ubiquinone by thin-layer chromatography as described previously (10). A ubiquinone standard (Q_8) on a control plate had an R_f value of 0.19 and displayed a peak absorbance at 274 nm when eluted into ethanol. PR50 produced a strong signal which had an R_f value of 0.20 in this system and a peak absorbance at 273 nm in ethanol. In contrast, extracts of PR11 *aarE1* produced no detectable ubiquinone, and intermediates were not detected. These results are consistent with PR11 containing a mutation in the *ubiA* gene.

To determine if the *aarE* and *ubiA* genes are functionally equivalent, we conducted an unbiased search for *E. coli* genes that could complement the *aarE1* allele in *P. stuartii* PR11. An *E. coli* genomic library of partial *Sau*3AI fragments constructed in pET21a (kindly provided by P. deBoer, Case Western Reserve University) was introduced into PR11 (*aarE1*), and complementing clones were identified as described previously for pAFM11. Analysis of all complementing plasmids revealed a common insert, and one plasmid with an insert of approximately 3 kb was used in further studies. The insert in this plasmid was shown to hybridize to Kohara phages IF8 and 12B4 (17), both of which are predicted to have the *E. coli* region containing *plsB* and *ubiCA*. Therefore, based on the published sequence of *ubiCA* (28, 38), a 1,031-bp *Sma*I-*Bcl*I fragment predicted to contain only the *ubiA* gene was subcloned into pBC KS(−), resulting in pBC.ubiA. Sequence analysis of the ends of pBC.ubiA verified that it contained only the *ubiA* gene. Introduction of pBC.ubiA into PR11 (*aarE1*) resulted in complementation of the *aarE1* mutation, as indicated by the wild-type growth rate and the ability of the transformant to grow on succinate minimal medium (Table 1). As expected, pSK.aarE also restored the ability of PR11 to grow on succinate plates (Table 1). In addition, both pSK.aarE and pBC.ubiA were able to complement an *E. coli ubiA* mutant (HW273) (data not shown). Thus, *aarE* and *ubiA* are functionally equivalent.

Construction and characterization of an *aarE*::Km null allele. To confirm that the loss of *aarE* function was responsible for the observed phenotypes in PR11, an *aarE*::Km disruption

TABLE 1. Growth phenotypes

Strain	Growth on M9 medium with[a]:	
	Glucose	Succinate
PR50 (wild type)	+	+
PR11 (aarE1)	+	−
PR11/pBluescript II SK(−)	+	−
PR11/pSK.aarE	+	+
PR11/pBC KS(−)	+	−
PR11/pBC.ubiA	+	+
PR11.D (aarE2::Km)	+	−
PR11.D/pBluescript II SK(−)	+	−
PR11.D/pSK.aarE	+	+
PR11.D/pBC KS(−)	+	−
PR11.D/pBC.ubiA	+	+

[a] Strains were grown on M9 minimal agar plates containing either 0.2% glucose or 0.5% succinate. +, wild-type growth rate; −, no growth.

was constructed by allelic replacement. A kanamycin resistance cassette, obtained as a 1.4-kb SmaI fragment from pUC4.KIXX (Pharmacia), was inserted at codon 148, in the middle of the aarE coding region, at a unique NarI site that had been blunt ended with the Klenow fragment of DNA polymerase I and deoxynucleoside triphosphates. A BamHI-ApaI fragment containing the aarE::Km disruption and flanking DNA was then excised and cloned into the suicide plasmid pKNG101 (16) cut with the same enzymes, resulting in pKNG101.aarE::Km. The chromosomal copy of aarE was disrupted by procedures which have been previously described (21, 22, 33, 35). Colonies with the aarE2::Km disruption were identified by their kanamycin resistance and small-colony phenotype. Southern blot analysis of three colonies with this phenotype, using probes for both aarE and the kanamycin resistance cassette, indicated the presence of the aarE2::Km disruption (data not shown).

Strain PR11.D (aarE2::Km) displayed a phenotype indistinguishable from that of PR11 (aarE1) with respect to growth rate and the inability to grow on succinate minimal medium (Table 1). The introduction of plasmid pSK.aarE or pBC.ubiA into PR11.D restored both the wild-type growth rate and the ability to grow on succinate minimal medium (Table 1). In addition, Northern blot analysis demonstrated that the accumulation of aac(2')-Ia mRNA was severely decreased in PR11.D (aarE2::Km) relative to the wild-type strain PR50 (data not shown).

Concluding remarks. Loss-of-function mutations in the aarE gene of P. stuartii resulted in high-level aminoglycoside resistance. The AarE gene product was 61% identical and functionally equivalent to the UbiA protein of E. coli. UbiA is an octaprenyltransferase which catalyzes the second step in ubiquinone biosynthesis, the addition of a prenyl group to 4-hydroxybenzoate (38, 41). Thus, in a ubiA mutant, ubiquinone biosynthesis is blocked, leading to a defect in electron transport and aerobic respiration. This results in the slow-growth phenotype observed in the aarE (ubiA) mutants and also explains the high-level aminoglycoside resistance that is independent of aac(2')-Ia. A number of previous studies have established that high-level aminoglycoside resistance can result from alterations in electron transport (1–5, 13, 14, 22, 25, 27, 36a), including a deficiency in ubiquinone (3, 4, 27).

A potentially important result from this study, in combination with our analysis of a new locus involved in ubiquinone biosynthesis (23), is the observation that mutants unable to produce ubiquinone have altered levels of aac(2')-Ia mRNA.

The regulatory mechanism(s) which requires ubiquinone or a functional electron transport chain to maintain normal levels of aac(2')-Ia mRNA accumulation remains to be identified. The decreased aac(2')-Ia mRNA accumulation is probably not due to a general defect in electron transport, since cydD mutants display increased aac(2')-Ia mRNA accumulation (22). In addition, the reduced aac(2')-Ia mRNA levels in the aarE mutant background are probably not due to a reduced growth rate. Mutations in the aarB, aarC, and aarD genes all reduce the growth rate to a level similar to that of an aarE mutant, yet these mutations lead to 3- to 12-fold increases in aac(2')-Ia mRNA accumulation relative to the wild type (22, 34, 35). Studies using an aac(2')-lacZ fusion in a single copy indicate that the accumulation of β-galactosidase is similar in the wild type and aarE::Km mutants (32). Based on the above-described data, the decrease in aac(2')-Ia mRNA accumulation in the aarE (ubiA) mutants appears to result from decreased mRNA stability. This could result from changes in the cellular levels of an RNase.

Nucleotide sequence accession number. The aarE nucleotide sequence has been deposited in GenBank under accession no. AF036909.

REFERENCES

1. **Bryan, L. E., and H. M. Van Den Elzen.** 1976. Streptomycin accumulation in susceptible and resistant strains of Escherichia coli and Pseudomonas aeruginosa. Antimicrob. Agents Chemother. **9:**928–938.
2. **Bryan, L. E., and S. Kwan.** 1981. Aminoglycoside-resistant mutants of Pseudomonas aeruginosa deficient in cytochrome d, nitrate reductase, and aerobic transport. Antimicrob. Agents Chemother. **19:**958–964.
3. **Bryan, L. E., and S. Kwan.** 1983. Roles of ribosomal binding, membrane potential, and electron transport in bacterial uptake of streptomycin and gentamicin. Antimicrob. Agents Chemother. **23:**835–845.
4. **Bryan, L. E., and H. M. Van Den Elzen.** 1977. Effects of membrane-energy mutations and cations on streptomycin and gentamicin accumulation by bacteria: a model for entry of streptomycin and gentamicin in susceptible and resistant bacteria. Antimicrob. Agents Chemother. **12:**163–177.
5. **Campbell, B. D., and R. J. Kadner.** 1980. Relation of aerobiosis and ionic strength to the uptake of dihydrostreptomycin in Escherichia coli. Biochim. Biophys. Acta **593:**1–10.
6. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134:**1141–1156.
7. **Chevereau, M., P. J. L. Daniel, J. Davies, and F. LeGoffic.** 1974. Aminoglycoside resistance in bacteria mediated by gentamicin acetyltransferase II, an enzyme modifying the 2'-amino group of aminoglycoside antibiotics. Biochemistry **13:**1141–1156.
8. **Clarke, A. J.** 1993. Extent of peptidoglycan O acetylation in the tribe Proteeae. J. Bacteriol. **175:**4550–4553.
9. **Clarke, A. J., and C. Dupont.** 1991. O-Acetylated peptidoglycan: its occurrence, pathobiological significance, and biosynthesis. Can. J. Microbiol. **38:**85–91.
10. **Collins, M. D.** 1985. Analysis of isoprenoid quinones. Methods Microbiol. **18:**329–366.
11. **Cox, G. B., and J. A. Downie.** 1979. Isolation and characterization of mutants of Escherichia coli K-12 affected in oxidative phosphorylation or quinone biosynthesis. Methods Enzymol. **56:**106–117.
12. **Cox, G. B., I. G. Young, L. M. McCann, and F. Gibson.** 1969. Biosynthesis of ubiquinone in Escherichia coli K-12: location of genes affecting the metabolism of 3-octaprenyl-4-hydroxybenzoic acid and 2-octaprenylphenol. J. Bacteriol. **99:**450–458.
13. **Damper, P. D., and W. Epstein.** 1981. Role of the membrane potential in bacterial resistance to aminoglycoside antibiotics. Antimicrob. Agents Chemother. **20:**803–808.
14. **Eisenberg, E. S., L. J. Mandel, H. R. Kaback, and M. H. Miller.** 1984. Quantitative association between electrical potential across the cytoplasmic membrane and early gentamicin uptake and killing in Staphylococcus aureus. J. Bacteriol. **157:**863–867.
15. **Howlett, B. J., and J. Bar-Tana.** 1980. Polyprenyl p-hydroxybenzoate carboxylase in flagellation of Salmonella typhimurium. J. Bacteriol. **143:**644–651.
16. **Kaniga, K., I. Delor, and G. Cornelis.** 1991. A wide-host-range suicide vector for improving reverse genetics in gram-negative bacteria: inactivation of the

blaA gene of *Yersinia enterocolitica*. Gene **109:**137–141.

17. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:**495–508.

18. **Lawrence, J., G. B. Cox, and F. Gibson.** 1974. Biosynthesis of ubiquinone in *Escherichia coli* K-12: biochemical and genetic characterization of a mutant unable to convert chorismate into 4-hydroxybenzoate. J. Bacteriol. **118:**41–45.

19. **Lee, P. T., A. Y. Hsu, H. T. Ha, and C. F. Clarke.** 1997. A *C*-methyltransferase involved in both ubiquinone and menaquinone biosynthesis: isolation and identification of the *Escherichia coli ubiE* gene. J. Bacteriol. **179:**1748–1754.

20. **Lightner, V. A., R. M. Bell, and P. Modrich.** 1983. The DNA sequences encoding *plsB* and *dgk* loci of *Escherichia coli*. J. Biol. Chem. **258:**10856–10861.

21. **Macinga, D. R., M. M. Parojcic, and P. N. Rather.** 1995. Identification and analysis of *aarP*, a transcriptional activator of the 2′-*N*-acetyltransferase in *Providencia stuartii*. J. Bacteriol. **177:**3407–3413.

22. **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.

23. **Macinga, D. R., G. M. Cook, R. K. Poole, and P. N. Rather.** 1998. Identification and characterization of *aarF*, a locus required for production of ubiquinone in *Providencia stuartii* and *Escherichia coli* and for expression of 2′-*N*-acetyltransferase in *Providencia stuartii*. J. Bacteriol. **180:**128–135.

24. **Meganathan, R.** 1996. Biosynthesis of the isprenoid quinones menaquinone (vitamin K_2) and ubiquinone (coenzyme Q), p. 642–656. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. ASM Press, Washington, D.C.

25. **Miller, M. H., S. C. Edberg, L. J. Mandel, C. F. Behar, and N. H. Steigbigel.** 1980. Gentamicin uptake in wild-type and aminoglycoside-resistant small-colony mutants of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **18:**722–729.

26. **Miller, V. L., and J. J. Mekalanos.** 1988. A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR*. J. Bacteriol. **170:**2575–2583.

27. **Muir, M. E., D. R. Hanwell, and B. J. Wallace.** 1981. Characterization of a respiratory mutant of *Escherichia coli* with reduced uptake of aminoglycoside antibiotics. Biochim. Biophys. Acta **638:**234–241.

28. **Nichols, B. P., and J. M. Green.** 1992. Cloning and sequencing of *Escherichia coli ubiC* and purification of chorismate lyase. J. Bacteriol. **174:**5309–5316.

29. **Payie, K. G., P. N. Rather, and A. J. Clarke.** 1995. Contribution of gentamicin 2′-*N*-acetyltransferase to the O acetylation of peptidoglycan in *Providencia stuartii*. J. Bacteriol. **177:**4303–4310.

30. **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.

31. **Poole, R. K., H. D. Williams, J. A. Downie, and F. Gibson.** 1989. Mutations affecting the cytochrome *d*-containing oxidase complex of *Escherichia coli* K-12: identification and mapping of a fourth locus, *cydD*. J. Gen. Microbiol. **135:**1865–1874.

32. **Rather, P. N.** Unpublished results.

33. **Rather, P. N., and E. Orosz.** 1994. Characterization of *aarA*, a pleiotrophic negative regulator of the 2′-*N*-acetyltransferase in *Providencia stuartii*. J. Bacteriol. **176:**5140–5144.

34. **Rather, P. N., E. Orosz, K. J. Shaw, R. Hare, and G. Miller.** 1993. Characterization and transcriptional regulation of the 2′-*N*-acetyltransferase gene from *Providencia stuartii*. J. Bacteriol. **175:**6492–6498.

35. **Rather, P. N., K. A. Solinsky, M. R. Paradise, and M. M. Parojcic.** 1997. *aarC*, an essential gene involved in density-dependent regulation of the 2′-*N*-acetyltransferase in *Providencia stuartii*. J. Bacteriol. **179:**2267–2273.

36. **Stroobant, P., I. G. Young, and F. Gibson.** 1972. Mutants of *Escherichia coli* K-12 blocked in the final reaction of ubiquinone biosynthesis: characterization and genetic analysis. J. Bacteriol. **109:**134–139.

36a.**vonEiff, C., C. Heilmann, R. A. Proctor, C. Woltz, G. Peters, and F. Götz.** 1997. A site-directed *Staphylococcus aureus hemB* mutant is a small colony variant which persists intracellularly. J. Bacteriol. **179:**4706–4712.

37. **Wu, G., H. D. Williams, M. Zamanian, F. Gibson, and R. K. Poole.** 1992. Isolation and characterization of *Escherichia coli* mutants affected in aerobic respiration: the cloning and nucleotide sequence of *ubiG*. J. Gen. Microbiol. **138:**2101–2112.

38. **Wu, G., H. D. Williams, F. Gibson, and R. K. Poole.** 1993. Mutants of *Escherichia coli* affected in respiration: the cloning and nucleotide sequence of *ubiA*, encoding the membrane-bound *p*-hydroxybenzoate:octaprenyltransferase. J. Gen. Microbiol. **139:**1795–1805.

39. **Yamaguchi, M., and S. Mitsuhashi.** 1974. A 2′-*N*-acetylating enzyme of aminoglycosides. J. Antibiot. **27:**507–515.

40. **Young, I. G., L. M. McCann, P. Stroobant, and F. Gibson.** 1971. Characterization and genetic analysis of mutant strains of *Escherichia coli* K-12 accumulating the ubiquinone precursors 2-octaprenyl-6-methoxy-1,4-benzoquinone and 2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinone. J. Bacteriol. **105:**769–778.

41. **Young, I. G., R. A. Leppik, J. A. Hamilton, and F. Gibson.** 1972. Biochemical and genetic studies on ubiquinone biosynthesis in *Escherichia coli* K-12: 4-hydroxybenzoate octaprenyltransferase. J. Bacteriol. **110:**18–25.