Characterization of the Na⁺-transporting NADH:ubiquinone oxidoreductase (NQR) in *Vibrio cholerae*

Sara R. Fassio[1], Yusuke Minato[2], Matthew J. Quinn[1], Wyatt J. Faulkner[1] and Claudia C. Häse[1,2]

[1]Department of Microbiology, [2]Department of Biomedical Sciences, Oregon State University

College of Veterinary Medicine

Abstract

We previously reported that inhibition of the Na⁺ translocating NADH:ubiquinone oxidoreductase (NQR), either by chemical inhibition or mutation, increased *toxT* transcription in *Vibrio cholerae*. In this study, we revealed that the *nqr* mutant strain showed similar phenotypes as the *Escherichia coli* NADH dehydrogenase I (*nuo*) mutant strain (e.g. growth defect after the mid log growth phase and higher acetic acid production). The increased growth and *toxT* expression in the *nqr* mutant relative to the wild type strain appears to be growth phase dependent. However, after longer growth, nqr showed lower amounts of cholera toxin production compared to the parent strain. Interestingly, the *nqr* mutant strain showed a similar level of *toxT* expression in the presence of L-lactate, which is known to stimulate respiration. Through Biolog® Phenotype Microarray (PM) analysis, we found that the *V. cholerae nqr* mutant strain had defects in a broad spectrum of metabolism functions, including amino acid, carboxylic acid, phosphorus, and sulfur utilization, indicating an important role of NQR in *V. cholerae* metabolism. In addition, *nqr* mutation increased osmotic sensitivity in *V. cholerae*. Some of the defects identified by PM analysis, including NaCl sensitivity, were restored by the addition of L-lactate.

Background

The Na⁺ translocating NADH:ubiquinone oxidoreductase (NQR) is an unique respiratory enzyme catalyzing the electron transfer from NADH to quinone, coupled with the translocation of sodium ions across the membrane. A genomic analysis of *Vibrio cholerae* revealed that it does not possess an ortholog of Complex I of the electron transport chain, NADH:ubiquinone oxidoreductase (NUO), which often acts as the main respiratory NADH dehydrogenase in bacteria (1). This suggests NQR plays an important role in energy metabolism in *V. cholerae*. We previously reported that the inactivation of NQR either by chemical inhibition or mutation increased virulence gene expression in *V. cholerae* (2). However, the details of the mechanisms of the link between NQR and virulence gene expression are still unclear.

Conclusions

- Lack of functional NQR affects wide ranges of *V. cholerae* metabolism, suggesting an important role for NQR in *V. cholerae* metabolism.
- The effects of NQR on virulence gene expression are growth phase dependent.
- We hypothesize that the changes in certain metabolic functions are linked to the observed changes in virulence gene expression following loss of NQR in *V. cholerae*.

References

1. **Heidelberg, J. F., J. A. Eisen, W. C. Nelson, R. A. Clayton, M. L. Gwinn, R. J. Dodson, D. H. Haft, E. K. Hickey, J. D. Peterson, L. Umayam, S. R. Gill, K. E. Nelson, T. D. Read, H. Tettelin, D. Richardson, M. D. Ermolaeva, J. Vamathevan, S. Bass, H. Y. Qin, I. Dragoi, P. Sellers, L. McDonald, T. Utterback, R. D. Fleishmann, W. C. Nierman, O. White, S. L. Salzberg, H. O. Smith, R. R. Colwell, J. J. Mekalanos, J. C. Venter, and C. M. Fraser.** 2000. DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. Nature **406**:477-483.
2. **Häse, C. C., and J. J. Mekalanos.** 1999. Effects of changes in membrane sodium flux on virulence gene expression in *Vibrio cholerae*. Proc Natl Acad Sci U S A **96**:3183-7.

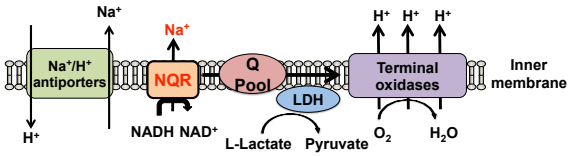

Fig. 1. NQR in the aerobic respiration system in *V. cholerae*.

The electron transport system of *V. cholerae* possesses a redox driven sodium pump (NQR) instead of the NADH:ubiquinone oxidoreductase (NUO) found in *E. coli* and other bacteria.
LDH: lactate dehydrogenase, Q: quinone

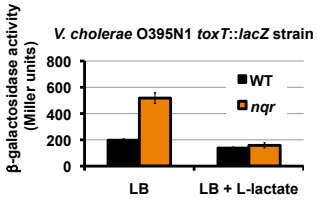

Fig. 3. Effects of L-lactate on *toxT* transcription.

The *nqr* mutant strain did not show increased *toxT* transcription in the presence of L-lactate.

Table 1. Biolog® Phenotype Microarray analysis of the *V. cholerae nqr* mutant.

Mode of action	Substrates
C-Source	L-Aspartic acid, L-Proline, L-Histidine, L-Glutamine, L-Glutamic acid, Gly-Glu Acetic acid, D,L-Malic acid, Fumaric acid Propionic acid, Succinic acid, L-Malic acid Mono-Methylsuccinate, Succinamic acid
S-Source	Glutathione
Osmotic sensitivity	1% NaCl, 5% Ethylene Glycol, 2% Sodium Sulfate, 2% Urea

Phenotype microarray analysis of the *V. cholerae nqr* mutant indicated defects in broad ranges of amino acid and carboxylic acid metabolism and osmotic sensitivity. Substrates in red were confirmed by growth-based assays.

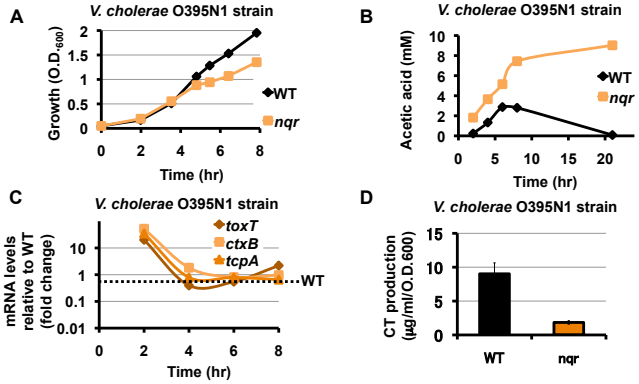

Fig. 2. Effects of NQR mutation on growth (A), acetate secretion (B), virulence gene expression (C), and cholera toxin production (D).

(A) The *nqr* mutant growth was similar to the wild type strain at early log growth phase but showed a growth defect after mid-log growth phase. (B) The *nqr* mutant showed higher acetate excretion compared to the wild type. (C) The *nqr* mutant showed higher virulence gene expression levels only at early log growth phase. (D) The *nqr* mutant produced lower cholera toxin levels compared to the wild type when measured after 16 hr growth in LB at 30ºC.

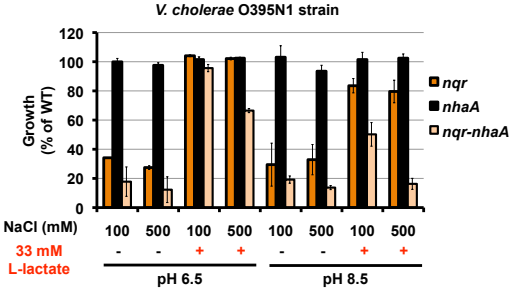

Fig. 4. Affect of L-Lactate on *V. cholerae* growth in high NaCl media.

In the presence of L-lactate, the *nqr* mutant strain grew similarly to wild type levels, even in the presence of high NaCl (500 mM). The results indicate that the combination of NQR and the Na⁺/H⁺ antiporter, NhaA, is essential for NaCl resistance, especially at pH 8.5.