Vibrio vulnificus: a physiological and genetic approach to the viable but nonculturable response

Abstract In this review, we focus on studies of the viable but nonculturable response (VBNC) of Vibrio vulnificus, a significant and aggressive human pathogen, as a model system for the general understanding of the VBNC response. This response is characterized physiologically as the inability to culture an organism on media that normally supports its growth, and yet those cells retain indicators of metabolic activity. Implicit in this definition is that it may be possible to return or resuscitate VBNC cells to active division on laboratory media. Since its original description in 1985, the VBNC response has been recognized in a range of bacteria. Study of the VBNC response has traditionally focused on physiological methods aimed at demonstrating that VBNC cells are indeed viable but have a specific block that prevents them from dividing on laboratory media, and such study has attempted to identify conditions that unequivocally demonstrate the resuscitation of VBNC cells. With the advent of molecular genetics, VBNC studies have begun to focus on genetics as a means to determine whether there are specific genes or regulatory pathways responsible for the development of the VBNC response. Thus, by combining information from physiological and genetic experiments, it is hoped that it can be determined whether the VBNC response represents a genetically programmed physiological adaptation similar to sporulation and outgrowth or whether VBNC represents the slow loss of function on the way to cellular death.

Key words VBNC · Vibrio vulnificus · Adaptive response · Starvation · Resuscitation · Marine bacteria

Introduction

Vibrio vulnificus is a particularly intriguing bacterium to study, not only because it is an important human pathogen but also because it has a series of physiological responses and adaptive strategies which it utilizes to optimize its survival under stressful conditions. In a clinical setting, V. vulnificus is one of the most potent and rapidly lethal bacterial pathogens described, leading to mortality in 60% of susceptible patients. Pathogenesis is clearly linked to iron availability (siderophore production), capsule production, and metalloprotease production, as well as the production of a large number of proteases, including, but not limited to, elastase, collagenase, neuraminidase, and lipase. The treatment of a V. vulnificus infection requires aggressive antibiotic therapy if the infection is caught early on, or in the case of severe wound infections, amputation or tissue debridement may be required to limit the tissue necrosis. V. vulnificus is commonly contracted by the consumption of raw shellfish, especially oysters, which accumulate the bacterium. Infection can also occur through the contact of seawater with an open wound, even one as small as an ant bite, which indicates that the organism is present as a natural member of the marine environment. As a pathogen, the mechanisms and treatments of a V. vulnificus infection are quite clear. However, in its natural setting, the survival and persistence of V. vulnificus is more enigmatic and less clearly understood. What are its reservoirs and what is its precise niche in the marine environment? Early observations off the East Coast of the United States determined that V. vulnificus could be isolated almost ubiquitously from the water column and sediments in the warmer months, but in the cooler months the organism was undetectable. Further investigation into the temporal distribution of V. vulnificus demonstrated that the number of culturable V. vulnificus in the water column, sediment, and oyster tissue...
declined in the cooler months. Those observations led to the hypothesis that *V. vulnificus* was not truly absent from marine waters in the cooler months, but, rather, was in a non-platable, or nonculurable, form. Indeed, these researchers went on to determine that *V. vulnificus* persisted in those sites in a viable but nonculurable (VBNC) state and that low temperature induced the VBNC response, which, in turn, accounted for the established temporal distribution of the organism. For the purposes of this review, VBNC cells are defined as those cells which retain the capacity to establish cellular division on media which normally support non-platable, or nonculturable, form. Indeed, these marine waters in the cooler months, but, rather, was in a non-culturable response when presented with a particular stress, and this response is presumed to protect the cells from that stress. However, entry into the VBNC state seems to be more broadly protective, as VBNC cells have also been demonstrated to have increased resistance to other stresses, such as oxidative damage, mechanical disruption, and heat shock. Thus, VBNC is particularly relevant because it may represent a widely distributed mechanism for bacterial survival in sub-optimal conditions. Indeed, the VBNC response may be a general survival mechanism analogous to the sporulation of some bacteria, including *Bacillus*, *Streptomyces*, and the Myxobacteria. For these organisms, conditions of stress initiate a specific developmental program which leads to the formation of highly resistant forms of the cells, spores, which, under favorable conditions, can develop into vegetative cells and reinitiate active growth. By analogy, stressful conditions encountered by some non-sporulating organisms lead to the VBNC response which protects the cells, and when conditions become more favorable, the cells may recover and reinitiate active growth. In particular, it is the recovery or resuscitation from the VBNC state to one of active growth that represents the completion of the developmental program. It also follows that if the VBNC response is truly a developmental program, then there should also be a series of genetically regulated steps which are involved in the progression of that response. Thus, methods directed at the identification of genetic elements involved in the development of the VBNC response or subsequent resuscitation may be particularly interesting and useful for studies of this response.

What follows is a discussion of the VBNC response and current research which is aimed at addressing the VBNC response at the physiological and genetic levels. The aim herein is to discuss some of the methods used for the assessment of the VBNC response, and the strengths and weaknesses of those methods; to discuss potential new avenues for studies of this response; and finally, to present recent advances in understanding the VBNC response. For the purposes of this review, we will focus on *V. vulnificus*, with the understanding that it is presented as a model organism for the general understanding of the VBNC response.

### Methods

There have been many suggestions for, and development of, methods used to distinguish whether nonculurable cells are indeed metabolically competent and thus viable, or whether they are merely moribund cells on the way to death. The methods for assessment of viability range from those that monitor physiological activity to the maintenance of cellular structures to genetic methods. Positive responses in these assays indicate that a portion of the nonculurable population is capable of performing complex cellular functions indicative of active or living cells. Each of the methods used to distinguish whether nonculurable organisms lead to the VBNC response may be particularly interesting and useful for studies of this response.