

Review Article

Viable but Nonculturable Bacteria: Food Safety and Public Health Perspective

Md. Fakruddin,¹ Khanjada Shahnewaj Bin Mannan,² and Stewart Andrews³

¹ Industrial Microbiology Laboratory, Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh

² Center for Food and Waterborne Diseases, icddr, b, Dhaka, Bangladesh

³ Oakden-Osmosis, Australia

Correspondence should be addressed to Md. Fakruddin; fakruddinmurad@gmail.com

Received 7 August 2013; Accepted 1 September 2013

Academic Editors: H.-P. Horz, R. E. Levin, and J. L. McKillip

Copyright © 2013 Md. Fakruddin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The viable but nonculturable (VBNC) state is a unique survival strategy of many bacteria in the environment in response to adverse environmental conditions. VBNC bacteria cannot be cultured on routine microbiological media, but they remain viable and retain virulence. The VBNC bacteria can be resuscitated when provided with appropriate conditions. A good number of bacteria including many human pathogens have been reported to enter the VBNC state. Though there have been disputes on the existence of VBNC in the past, extensive molecular studies have resolved most of them, and VBNC has been accepted as a distinct survival state. VBNC pathogenic bacteria are considered a threat to public health and food safety due to their nondetectability through conventional food and water testing methods. A number of disease outbreaks have been reported where VBNC bacteria have been implicated as the causative agent. Further molecular and combinatorial research is needed to tackle the threat posed by VBNC bacteria with regard to public health and food safety.

1. Introduction

The cells that form a colony on specific nutrient media are the culturable cells. Viable means metabolically or physiologically active. So the cells that are metabolically or physiologically active but cannot be cultured on specific media are the viable but nonculturable cells (VBNC) [1]. Most microorganisms growing in nature have yet to be cultured in the laboratory. In fact, less than 1% of the microorganisms in natural water and soil samples are cultured in viable count procedures [2].

In 1982, Xu and coworkers introduced the term “viable but nonculturable bacterial cells (VBNC)” to distinguish particular cells that could not form colonies on solid media but retained metabolic activity and the ability to elongate after the administration of nutrients [3]. According to Oliver [4], “VBNC can be defined as a metabolically active bacterial cell that crossed a threshold in this way, for known or unknown reasons, and became unable to multiply in or on a medium normally supporting its growth.” Most of the bacteria that

enter VBNC state are gram-negative species belonging to the gamma subclass of the proteobacteria branch, except for *Rhizobium*, *Agrobacterium*, and *Helicobacter-Campylobacter* species [5].

2. History

Bashford and colleagues [6] announced that they had recovered *Vibrio cholerae* from streams and drainage ditches, including sites with negligible chance of sewage contamination. Around the same time, Colwell et al. [7] also found *Vibrio cholerae* in Maryland, USA. She and her coworkers showed that, in artificial sea water, *Vibrio cholera* and *E. coli* remained viable though they lost the capacity to form colonies on culture media [8]. Soon *Salmonella enteritidis*, *Shigella sonnei*, and *Legionella pneumophila* joined the list of organisms known to be capable of entering a state in which they failed to show up on nutrient agar yet took up substrates and signaled in other ways that they were

certainly not dead [9]. The use of laboratory media to recover and enumerate bacteria and to link them with or absolve them from pathological and other activities became obsolete by the new discoveries, and the term VBNC (viable but nonculturable) was introduced [10].

3. VBNC

Microorganisms that do not grow in culture media, but are still metabolically active and capable of causing infections in animals and plants, are said to be in a VBNC state. Traditional laboratory culture conditions and methods cannot meet the requirements of VBNC organisms to resume growth [11]. Semistarved bacteria usually resume growth immediately when appropriate nutrients and conditions are provided. Viable but nonculturable cells will not resume growth even when nutrients are provided [12]. VBNC cells exhibit active metabolism in the form of respiration or fermentation, incorporate radioactive substrates, and have active protein synthesis but cannot be cultured or grown on conventional laboratory media. They have been detected by observing discrepancies between plate count enumeration of bacterial population and direct staining and microscopic counts [13]. These cells may be a problem in the environment if they are pathogens. For example, viable but nonculturable cells of *Vibrio cholerae*, enteropathogenic *E. coli*, *Legionella pneumophila*, and various other bacteria have been shown to regain culturability after they have entered the intestinal tracts of animals [14].

The VBNC state is defined as a state of dormancy triggered by harsh environmental conditions [15], such as nutrient starvation [16], extreme temperatures [17], and sharp changes in pH or salinity [18]; osmotic stress [19], oxygen availability [20, 21], and damage to or lack of an essential cellular component including DNA; exposure to food preservatives [22] and heavy metals [23, 24]; exposure to white light [25]; activation of lysogenic phages or suicide genes such as *sok/hak* or autolysins [26]; and decontaminating processes such as pasteurization of milk [27] and chlorination of wastewater [28].

The characteristics of bacteria in the VBNC state can be summarized as follows:

- (i) maintaining apparent cell integrity;
- (ii) possessing some form of measurable cellular activity [29];
- (iii) possessing apparent capacity to regain culturability *in vivo* [30];
- (iv) responding to external stimulus as shown by specific gene expression [31];
- (v) having low metabolic activity [28];
- (vi) exhibiting dwarfing [32];
- (vii) having reduced nutrient transport;
- (viii) containing a high ATP level and exhibit high membrane potential [33];
- (ix) having extensive modifications to the fatty acid composition in cytoplasmic membranes [34];

- (x) within the cell wall peptidoglycan, increased crosslinking, increased muropeptides bearing covalently bound lipoprotein, and shortening of the average length of glycan strands [35];
- (xi) higher autolytic capability than exponentially growing cells;
- (xii) retained plasmids;
- (xiii) increased antibiotic resistance due to lower metabolic activity [15];
- (xiv) changes in outer-membrane protein profile [36];
- (xv) continuous gene expression [37].

The VBNC state continues to be disputed due to the difficulties of differentiating VBNC cells and dormant cells through resuscitation and phenotypic studies. However, recent molecular studies have generated data to support the existence of the VBNC state [38].

4. VBNC Pathogens

The VBNC is also considered an important reservoir of many human pathogens in the environment [39].

The following list includes but is not limited to the pathogenic bacteria that can enter the VBNC state [15]: *Aeromonas hydrophila*, *Agrobacterium tumefaciens*, *Burkholderia cepacia*, *Campylobacter jejuni*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Escherichia coli* (including EHEC), *Helicobacter pylori*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Streptococcus faecalis*, *Vibrio alginolyticus*, *Vibrio cholerae*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* (types 1 and 2).

5. Public Health Significance of VBNC

Many believe that pathogens in the VBNC state are unable to induce infection/disease despite retaining their virulent properties. However, when VBNC pathogens pass through a host animal [40], resuscitation and resumption of metabolic activity have led to infections and diseases [40, 41]. The first evidence of pathogenicity of nonculturable cells was the demonstration of fluid accumulation in the rabbit ileal loop assay (RICA) by VBNC *Vibrio cholerae* O1, followed by human volunteer experiments [42]. VBNC *E. coli* nonculturable cells were also reisolated after passaging through rabbit ileal loops 4 days after inoculation, and chick embryos died when injected with nonculturable cells of *Legionella pneumophila*, leading to the conclusion that VBNC pathogens remain potentially pathogenic. So, VBNC has a huge significance in public health care [43].

Many indicator bacteria and pathogenic bacteria which exist in aquatic habitats have been shown to have a VBNC state [38]. Water is routinely tested for such indicators and pathogens, and if they are not detected or enumerated at a concentration below guidelines, waters are deemed to be safe

for public consumption [44]. Therefore, where circumstances indicate possible presence of VBNC pathogens, additional molecular methodology needs to be used to reduce the risk of infective disease outbreaks [45].

Thus, food and environmental and clinical samples no longer can be considered free from pathogens if culturing yields negative results. For the general public, the presence of VBNC in water and food may be related to low-grade infections or the so-called aseptic infections. In many cases, the infections are incorrectly attributed to viruses since no bacteria were detected. For example, *Vibrio cholerae* O1 in the surface water have been shown to remain in nonculturable state. These water sources are used for domestic purpose regularly and pose a risk of infection [46]. When conditions are not favorable for growth, then it transforms to the nonculturable state in association with crustacean copepods. Persistence of *Vibrio cholerae* in water in the VBNC state is an important public health factor, since detection will not be successful if only conventional cultural methods are used [47].

Similarly, *Shigella* can undergo VBNC state in water but become a threat when they enter the human body [48]. Additional studies have indicated that a good number of pathogenic bacteria can survive food and water treatment processes and persist as well as retain virulence in processed food, pasteurized milk, potable water, and the environment [49]. One study demonstrated that recurrent urinary tract infections in many individuals were caused by uropathogenic *E. coli* cells which remained in VBNC state [50]. Furthermore, these VBNC cells demonstrated resistance to antibiotic treatment causing reinfection [51]. Other studies showed that uropathogenic VBNC *E. coli* retain enteropathogenicity as shown by continual production of enterotoxin [52]. Nilsson et al. [53] showed that VBNC *Helicobacter pylori* cells can express virulence factors such as *cagA*, *vacA*, and *vreA*.

6. VBNC State of Foodborne Bacteria: A Challenge in Food Safety

The presence of VBNC bacteria in food is well documented [54]. Food and its surrounding environment are a complex system, in which physiochemical characteristics (pH, a_w , and chemical composition) and environmental factors (storage temperature and time, decontamination treatments, and packaging under modified atmosphere) act simultaneously on contaminating bacteria leading to the VBNC state [55]. For example, it has been demonstrated that refrigerated pasteurized grapefruit juice induced the VBNC state in *E. coli* O157:H7 and *S. typhimurium* within 24 hours of incubation [56]. Again, Gunasekera et al. [27] reported that in pasteurized milk which has undergone thermal treatment, contaminating bacteria such as *E. coli* and *Pseudomonas putida* enter into the VBNC state but retain transcription and translation functions. Several foodborne outbreaks have been reported in Japan, where pathogens such as *Salmonella enterica* subsp. *enterica* [57] and *E. coli* O157 [58] in the VBNC state were responsible for outbreaks.

The VBNC is also critical in determining shelf life and microbial stability of food and beverages. For example, acetic

acid and lactic acid bacteria entered the VBNC state in wine as a consequence of lack of oxygen and presence of sulphites [59].

7. Methods of Detection of VBNC Bacteria

7.1. Bright Field Microscopy with Nalidixic Acid. Nalidixic acid (20–40 mg/L) is used to stop cell division. After exposure to nalidixic acid, viable cells continue to grow and will appear elongated, whereas the nonviable metabolically inactive cells will retain their original shape and size. The cells are then observed under a microscope. Viable cells will be seen as elongated, whereas VBNC/dormant cells will be seen as oval and large [60].

7.2. Fluorescent Microscopy. Various fluorescent staining procedures can be used to determine VBNC organisms. Frequently used stains are acridine orange, 4,6-diamino-2-phenyl indole (DAPI), fluorescein isothiocyanate (FITC), indophenyl-nitrophenyl-phenyltetrazolium chloride (INT), and 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) [61]. The mode of action of these dyes and the reactions observed are summarised in Table 1.

In recent years, a new differential staining assay, the *BacLight* Live/Dead assay, has been developed. The assay allows simultaneous counting total and viable (metabolically active) cells, by using two nucleic acid stains, that is, green-fluorescent SYTO 9 stain and red-fluorescent propidium iodide stain. SYTO 9 stains both live and dead bacteria, whereas propidium iodide penetrates only bacteria having damaged membranes. When used together, propidium iodide reduces SYTO 9 fluorescence in dead bacteria with damaged membranes resulting in red fluorescent cells, whereas the live bacteria will fluoresce green [62].

7.3. Molecular Techniques. Hybridization probes are nucleic acids (DNA/RNA) which have been chemically or radioactively labeled and are used to detect complementary target DNA/RNA. Specific amplification of DNA targets in bulk DNA extracts from environmental and clinical samples permits detection of specific organisms or groups of related organisms without the need to cultivate them, provided the appropriate unique primers are used [63]. These procedures do not discriminate between culturable and nonculturable forms of the target organisms. Due to the failure of distinguishing between dead or live cells by DNA-based methods, RNA-based methods are a more valuable estimate of gene expression and/or cell viability under different conditions [64]. This technique is more able to discriminate between culturable and nonculturable forms of an organism. Furthermore, reverse transcriptase PCR (RT-PCR) can distinguish between live and dead cells. This is possible because it is an mRNA-based method and mRNA is short-lived (half-life less than 1 minute). Messenger RNA is only present in metabolically active cells and not found in nature after cell death. RT PCR can detect nonculturable but active or live cells [65].

TABLE 1: Fluorescent dyes used for detection of VBNC bacteria.

Dye	Mechanism	Reaction
Acridine orange	The staining response depends on the ratio of DNA to protein in the cells	Actively reproducing cells appear green but slow-growing or nonreproducing cells at time of staining appear orange
Di-amino-phenyl-indole (DAPI)	Differential staining	Living cells look green under fluorescent microscope
Indophenyl-nitrophenyl-phenyl tetrazolium chloride (INT)	INT reacts with dehydrogenase enzyme to produce formazone and red color, thus living cells appear red	Living cells appear red
Fluorescein isothiocyanate (FITC)	Enzyme activity in living cell	FITC stains living cells violet or blue

Even though traditional culture methods fail to detect the presence of specific VBNC in a sample, the presence of these microbes can be demonstrated using some of the molecular techniques described. More specifically, oligonucleotide probes of 18–20 nucleotides are proving most useful because they hybridize rapidly to specific DNA sequences of target organisms. These gene probes can reveal closely related organisms or organisms with similar functional capabilities. Additional molecular techniques are then required to fully identify any VNBC detected [66]. The detection of VBNC cells directly from the environmental samples can also be achieved using different types of blotting such as colony blot, slot blot, dot blot, and southern blot. The principle of blotting is the use of radio- or nonradioactive or fluorescence labeled probe. Fluorescent in situ Hybridization (FISH) is an alternative format for hybridization probes in which fluorescence labeled DNA or RNA probes are hybridized with target nucleic acids in whole, permeabilized cells [17]. The application of this method to the detection of single microbial cells by using rRNA-targeted probes in combination with epifluorescent microscopy has been developed. This is done through selective targeting of regions of rRNA, which consist of conserved and variable nucleotide regions. By choosing the appropriate rRNA probe sequence, FISH can be used to detect all bacterial cells (a universal probe) or a single population of cells (a strain-specific probe) of VBNC. It has lower sensitivity and cannot distinguish live and dead cells [63].

8. Conclusion

From the above discussions, it is evident that a number of nonspore-forming human pathogenic bacteria can enter the VBNC state with maintained cellular structure and biology and persistent gene expression but remain nonculturable by traditional cultural techniques. They can survive and revert to culturable conditions when provided with appropriate conditions, hence, being a significant threat to public health and food safety. Further research is needed to elucidate mechanisms leading to the VBNC and the development of methodologies to confirm their existence.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

- [1] G. Bogosian and E. V. Bourneuf, "A matter of bacterial life and death," *EMBO Reports*, vol. 2, no. 9, pp. 770–774, 2001.
- [2] I. Barcina and I. Arana, "The viable but nonculturable phenotype: a crossroads in the life-cycle of non-differentiating bacteria?" *Reviews in Environmental Science and Biotechnology*, vol. 8, no. 3, pp. 245–255, 2009.
- [3] H. S. Xu, N. Roberts, F. L. Singleton, R. W. Attwell, D. J. Grimes, and R. R. Colwell, "Survival and viability of nonculturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment," *Microbial Ecology*, vol. 8, no. 4, pp. 313–323, 1982.
- [4] J. D. Oliver, "The viable but non-culturable state in the human pathogen *Vibrio vulnificus*," *FEMS Microbiology Letters*, vol. 133, no. 3, pp. 203–208, 1995.
- [5] J. D. Oliver, "The public health significance of viable but nonculturable bacteria," in *Nonculturable Microorganisms in the Environment*, R. R. Colwell and D. J. Grimes, Eds., pp. 277–299, ASM Press, Washington, DC, USA, 2000.
- [6] D. J. Bashford, T. J. Donovan, A. L. Furniss, and J. V. Lee, "*Vibrio cholerae* in Kent," *The Lancet*, vol. 1, no. 8113, pp. 436–437, 1979.
- [7] R. R. Colwell, R. J. Seidler, J. Kaper et al., "Occurrence of *Vibrio cholerae* serotype O1 in Maryland & Louisiana estuaries," *Applied and Environmental Microbiology*, vol. 41, no. 2, pp. 555–558, 1981.
- [8] R. R. Colwell and D. J. Grimes, "Semantics and strategies," in *Nonculturable Microorganisms in the Environment*, R. R. Colwell and D. J. Grimes, Eds., pp. 1–6, ASM Press, Washington, DC, USA, 2000.
- [9] L. Sinton, "Viable but non-culturable bacteria—menace or myth?" *New Zealand Water & Wastes Association Journal*, vol. 143, pp. 31–38, 2006.
- [10] D. McDougald, S. A. Rice, D. Weichart, and S. Kjelleberg, "Non-culturable: adaptation or debilitation?" *FEMS Microbiology Ecology*, vol. 25, no. 1, pp. 1–9, 1998.
- [11] H. Yamamoto, "Viable but nonculturable state as a general phenomenon of non-sporeforming bacteria, and its modeling," *Journal of Infection and Chemotherapy*, vol. 6, no. 2, pp. 112–114, 2000.
- [12] T. Nyström, "Not quite dead enough: on bacterial life, culturability, senescence, and death," *Archives of Microbiology*, vol. 176, no. 3, pp. 159–164, 2001.
- [13] R. Sachidanandham and K. Yew-Hoong Gin, "A dormancy state in nonspore-forming bacteria," *Applied Microbiology and Biotechnology*, vol. 81, no. 5, pp. 927–941, 2009.
- [14] R. R. Colwell, P. Brayton, D. Herrington, B. Tall, A. Huq, and M. M. Levine, "Viable but non-culturable *Vibrio cholerae* O1 revert

- to a cultivable state in the human intestine," *World Journal of Microbiology and Biotechnology*, vol. 12, no. 1, pp. 28–31, 1996.
- [15] J. D. Oliver, "Recent findings on the viable but nonculturable state in pathogenic bacteria," *FEMS Microbiology Reviews*, vol. 34, no. 4, pp. 415–425, 2010.
- [16] K. L. Cook and C. H. Bolster, "Survival of *Campylobacter jejuni* and *Escherichia coli* in groundwater during prolonged starvation at low temperatures," *Journal of Applied Microbiology*, vol. 103, no. 3, pp. 573–583, 2007.
- [17] V. Besnard, M. Federighi, E. Declercq, F. Jugiau, and J.-M. Cappelier, "Environmental and physico-chemical factors induce VBNC state in *Listeria monocytogenes*," *Veterinary Research*, vol. 33, no. 4, pp. 359–370, 2002.
- [18] E. Cunningham, C. O'Byrne, and J. D. Oliver, "Effect of weak acids on *Listeria monocytogenes* survival: evidence for a viable but nonculturable state in response to low pH," *Food Control*, vol. 20, no. 12, pp. 1141–1144, 2009.
- [19] H. Asakura, K. Kawamoto, Y. Haishima, S. Igimi, S. Yamamoto, and S.-I. Makino, "Differential expression of the outer membrane protein W (OmpW) stress response in enterohemorrhagic *Escherichia coli* O157:H7 corresponds to the viable but non-culturable state," *Research in Microbiology*, vol. 159, no. 9–10, pp. 709–717, 2008.
- [20] B. D. Kana, B. G. Gordhan, K. J. Downing et al., "The resuscitation-promoting factors of *Mycobacterium tuberculosis* are required for virulence and resuscitation from dormancy but are collectively dispensable for growth in vitro," *Molecular Microbiology*, vol. 67, no. 3, pp. 672–684, 2008.
- [21] F. Mascher, C. Hase, Y. Moëgne-Loccoz, and G. Défago, "The viable-but-nonculturable state induced by abiotic stress in the biocontrol agent *Pseudomonas fluorescens* CHA0 does not promote strain persistence in soil," *Applied and Environmental Microbiology*, vol. 66, no. 4, pp. 1662–1667, 2000.
- [22] C. Quirós, M. Herrero, L. A. Garcáa, and M. Dáaz, "Quantitative approach to determining the contribution of viable-but-nonculturable subpopulations to malolactic fermentation processes," *Applied and Environmental Microbiology*, vol. 75, no. 9, pp. 2977–2981, 2009.
- [23] J. I. Ghezzi and T. R. Steck, "Induction of the viable but non-culturable condition in *Xanthomonas campestris* pv. *campestris* in liquid microcosms and sterile soil," *FEMS Microbiology Ecology*, vol. 30, no. 3, pp. 203–208, 1999.
- [24] R. del Campo, P. Russi, P. Mara et al., "*Xanthomonas axonopodis* pv. *citri* enters the VBNC state after copper treatment and retains its virulence," *FEMS Microbiology Letters*, vol. 298, no. 2, pp. 143–148, 2009.
- [25] M. Gourmelon, J. Cillard, and M. Pommepuy, "Visible light damage to *Escherichia coli* in seawater: oxidative stress hypothesis," *Journal of Applied Bacteriology*, vol. 77, no. 1, pp. 105–112, 1994.
- [26] E. Aizenman, H. Engelberg-Kulka, and G. Glaser, "An *Escherichia coli* chromosomal "addiction module" regulated by 3', 5'-bispyrophosphate: a model for programmed bacterial cell death," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 12, pp. 6059–6063, 1996.
- [27] T. S. Gunasekera, A. Sørensen, P. V. Attfield, S. J. Sørensen, and D. A. Veal, "Inducible gene expression by nonculturable bacteria in milk after pasteurization," *Applied and Environmental Microbiology*, vol. 68, no. 4, pp. 1988–1993, 2002.
- [28] J. D. Oliver, "The viable but nonculturable state in bacteria," *Journal of Microbiology*, vol. 43, pp. 93–100, 2005.
- [29] C.-J. Lai, S.-Y. Chen, I.-H. Lin, C.-H. Chang, and H.-C. Wong, "Change of protein profiles in the induction of the viable but nonculturable state of *Vibrio parahaemolyticus*," *International Journal of Food Microbiology*, vol. 135, no. 2, pp. 118–124, 2009.
- [30] A. M. Anuchin, A. L. Mulyukin, N. E. Suzina, V. I. Duda, G. I. El-Registan, and A. S. Kaprelyants, "Dormant forms of *Mycobacterium smegmatis* with distinct morphology," *Microbiology*, vol. 155, no. 4, pp. 1071–1079, 2009.
- [31] D. B. Kell, A. S. Kaprelyants, D. H. Weichart, C. R. Harwood, and M. R. Barer, "Viability and activity in readily culturable bacteria: a review and discussion of the practical issues," *Antonie van Leeuwenhoek*, vol. 73, no. 2, pp. 169–187, 1998.
- [32] K. Costa, G. Bacher, G. Allmaier et al., "The morphological transition of *Helicobacter pylori* cells from spiral to coccoid is preceded by a substantial modification of the cell wall," *Journal of Bacteriology*, vol. 181, no. 12, pp. 3710–3715, 1999.
- [33] C. Signoretto, M. D. M. Lleò, M. C. Tafi, and P. Canepari, "Cell wall chemical composition of *Enterococcus faecalis* in the viable but nonculturable state," *Applied and Environmental Microbiology*, vol. 66, no. 5, pp. 1953–1959, 2000.
- [34] A. P. Day and J. D. Oliver, "Changes in membrane fatty acid composition during entry of *Vibrio vulnificus* into the viable but nonculturable state," *Journal of Microbiology*, vol. 42, no. 2, pp. 69–73, 2004.
- [35] C. Signoretto, M. D. M. Lleò, and P. Canepari, "Modification of the peptidoglycan of *Escherichia coli* in the viable but nonculturable state," *Current Microbiology*, vol. 44, no. 2, pp. 125–131, 2002.
- [36] A. Muela, C. Seco, E. Camafeita et al., "Changes in *Escherichia coli* outer membrane subproteome under environmental conditions inducing the viable but nonculturable state," *FEMS Microbiology Ecology*, vol. 64, no. 1, pp. 28–36, 2008.
- [37] S. Maalej, M. Denis, and S. Dukan, "Temperature and growth-phase effects on *Aeromonas hydrophila* survival in natural seawater microcosms: role of protein synthesis and nucleic acid content on viable but temporarily nonculturable response," *Microbiology*, vol. 150, no. 1, pp. 181–187, 2004.
- [38] M. R. Barer and C. R. Harwood, "Bacterial viability and culturability," *Advances in Microbial Physiology*, vol. 41, pp. 93–137, 1999.
- [39] M. del Mar Lleò, D. Benedetti, M. C. Tafi, C. Signoretto, and P. Canepari, "Inhibition of the resuscitation from the viable but non-culturable state in *Enterococcus faecalis*," *Environmental Microbiology*, vol. 9, no. 9, pp. 2313–2320, 2007.
- [40] W. Baffone, B. Citterio, E. Vittoria et al., "Retention of virulence in viable but non-culturable halophilic *Vibrio* spp.," *International Journal of Food Microbiology*, vol. 89, no. 1, pp. 31–39, 2003.
- [41] Y. N. Sardessai, "Viable but non-culturable bacteria: their impact on public health," *Current Science*, vol. 89, p. 1650, 2005.
- [42] B. K.-N. Amel, B. Amine, and B. Amina, "Survival of *Vibrio fluvialis* in seawater under starvation conditions," *Microbiological Research*, vol. 163, no. 3, pp. 323–328, 2008.
- [43] J. M. Cappelier, V. Besnard, S. M. Roche, P. Velge, and M. Federighi, "Avirulent viable but non culturable cells of *Listeria monocytogenes* need the presence of an embryo to be recovered in egg yolk and regain virulence after recovery," *Veterinary Research*, vol. 38, no. 4, pp. 573–583, 2007.
- [44] C. Signoretto, G. Burlacchini, M. D. M. Lleò et al., "Adhesion of *Enterococcus faecalis* in the nonculturable state to plankton is the main mechanism responsible for persistence of this bacterium in both lake and seawater," *Applied and Environmental Microbiology*, vol. 70, no. 11, pp. 6892–6896, 2004.

- [45] B. Rivers and T. R. Steck, "Viable but nonculturable uropathogenic bacteria are present in the mouse urinary tract following urinary tract infection and antibiotic therapy," *Urological Research*, vol. 29, no. 1, pp. 60–66, 2001.
- [46] C. Edwards, "Problems posed by natural environments for monitoring microorganisms," *Molecular Biotechnology*, vol. 15, no. 3, pp. 211–223, 2000.
- [47] M. R. Barer, L. T. Gribbon, C. R. Harwood, and C. E. Nwoguh, "The viable but non-culturable hypothesis and medical bacteriology," *Reviews in Medical Microbiology*, vol. 4, no. 4, pp. 183–191, 1993.
- [48] S. S. Epstein, "Microbial awakenings," *Nature*, vol. 457, no. 7233, p. 1083, 2009.
- [49] R. Colwell, "Bacterial death revisited," in *Non-Culturable Microorganisms in the Environment*, R. R. Colwell and K. J. Grimes, Eds., pp. 325–342, ASM Press, Washington, DC, USA, 2000.
- [50] M. Anderson, D. Bollinger, A. Hagler et al., "Viable but nonculturable bacteria are present in mouse and human urine specimens," *Journal of Clinical Microbiology*, vol. 42, no. 2, pp. 753–758, 2004.
- [51] M. A. Mulvey, J. D. Schilling, and S. J. Hultgren, "Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection," *Infection and Immunity*, vol. 69, no. 7, pp. 4572–4579, 2001.
- [52] M. Pommepuy, M. Butin, A. Derrien, M. Gourmelon, R. R. Colwell, and M. Cormier, "Retention of enteropathogenicity by viable but nonculturable *Escherichia coli* exposed to seawater and sunlight," *Applied and Environmental Microbiology*, vol. 62, no. 12, pp. 4621–4626, 1996.
- [53] H.-O. Nilsson, J. Blom, W. A. Al-Soud, A. A. Ljungh, L. P. Andersen, and T. Wadström, "Effect of cold starvation, acid stress, and nutrients on metabolic activity of *Helicobacter pylori*," *Applied and Environmental Microbiology*, vol. 68, no. 1, pp. 11–19, 2002.
- [54] M. Ordax, E. G. Biosca, S. C. Wimalajeewa, M. M. López, and E. Marco-Noales, "Survival of *Erwinia amylovora* in mature apple fruit calyces through the viable but nonculturable (VBNC) state," *Journal of Applied Microbiology*, vol. 107, no. 1, pp. 106–116, 2009.
- [55] F. Sun, J. Chen, L. Zhong et al., "Characterization and virulence retention of viable but nonculturable *Vibrio harveyi*," *FEMS Microbiology Ecology*, vol. 64, no. 1, pp. 37–44, 2008.
- [56] M. S. Nicolò, A. Giofrè, S. Carnazza, G. Platania, I. D. Silvestro, and S. P. P. Guglielmino, "Viable but nonculturable state of foodborne pathogens in grapefruit juice: a study of laboratory," *Foodborne Pathogens and Disease*, vol. 8, no. 1, pp. 11–17, 2011.
- [57] H. Asakura, S.-I. Makino, T. Takagi et al., "Passage in mice causes a change in the ability of *Salmonella enterica* serovar *Oranienburg* to survive NaCl osmotic stress: resuscitation from the viable but non-culturable state," *FEMS Microbiology Letters*, vol. 212, no. 1, pp. 87–93, 2002.
- [58] S.-I. Makino, T. Kii, H. Asakura et al., "Does enterohemorrhagic *Escherichia coli* O157:H7 enter the viable but nonculturable state in salted salmon roe?" *Applied and Environmental Microbiology*, vol. 66, no. 12, pp. 5536–5539, 2000.
- [59] V. Millet and A. Lonvaud-Funel, "The viable but non-culturable state of wine micro-organisms during storage," *Letters in Applied Microbiology*, vol. 30, no. 2, pp. 136–141, 2000.
- [60] B. L. Adams, T. C. Bates, and J. D. Oliver, "Survival of *Helicobacter pylori* in a natural freshwater environment," *Applied and Environmental Microbiology*, vol. 69, no. 12, pp. 7462–7466, 2003.
- [61] A. Villarino, O. M. M. Bouvet, B. Regnault, S. Martin-Delautre, and P. A. D. Grimont, "Exploring the frontier between life and death in *Escherichia coli*: evaluation of different viability markers in live and heat- or UV-killed cells," *Research in Microbiology*, vol. 151, no. 9, pp. 755–768, 2000.
- [62] N. J. Rowan, "Defining established and emerging microbial risks in the aquatic environment: current knowledge, implications, and outlooks," *International Journal of Microbiology*, vol. 2011, Article ID 462832, 15 pages, 2011.
- [63] K. L. Josephson, C. P. Gerba, and I. L. Pepper, "Polymerase chain reaction detection of nonviable bacterial pathogens," *Applied and Environmental Microbiology*, vol. 59, no. 10, pp. 3513–3515, 1993.
- [64] M. M. Lleò, S. Pierobon, M. C. Tafi, C. Signoretto, and P. Canepari, "mRNA detection by reverse transcription-PCR for monitoring viability over time in an *Enterococcus faecalis* viable but nonculturable population maintained in a laboratory microcosm," *Applied and Environmental Microbiology*, vol. 66, no. 10, pp. 4564–4567, 2000.
- [65] S. R. Pai, J. K. Actor, E. Sepulveda, R. L. Hunter Jr., and C. Jagannath, "Identification of viable and non-viable *Mycobacterium tuberculosis* in mouse organs by directed RT-PCR for antigen 85B mRNA," *Microbial Pathogenesis*, vol. 28, no. 6, pp. 335–342, 2000.
- [66] S. Heim, M. D. M. Lleò, B. Bonato, C. A. Guzman, and P. Canepari, "The viable but nonculturable state and starvation are different stress responses of *Enterococcus faecalis*, as determined by proteome analysis," *Journal of Bacteriology*, vol. 184, no. 23, pp. 6739–6745, 2002.



Hindawi

Submit your manuscripts at
<http://www.hindawi.com>

