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Correlative Imaging of Culturable and Non-Culturable Bacteria

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When bacterial cells are exposed to a sublethal stress, they may undergo dramatic modification in both cell conformation and metabolism which may result in a non-culturable state (NC). The level and the nature of these modifications depend on the type of the inducer (stress) and possibly the bacteria strain. NC cells could be present in a pharmaceutical environment. Microorganisms are ubiquitous in the natural environment and can be readily carried into a controlled clean room or production line. Microbiological contaminants present in a production line are subjected to harsh conditions that may stimulate the contaminants allowing them to transform into the NC state which may have been neglected or omitted by a conventional growth based examination method. This study was conducted to determine if conditions for inducing the NC state could be established in our laboratory as assessed by any morphological changes observed by electron microscopy.

Strains of *Vibrio vulnificus* and *Escherichia coli* K12 were grown in Brain Heart Infusion broth at 30-35℃ for 4-6 hours. The optical density (OD_{600nm}) of each culture was in the range from 0.1 to 0.3, indicating the bacterial growth of each strain is in exponential phase. The cell culture of each strain was then washed by centrifugation (3,000 x g for 15 min) twice and the pellets were suspended in saline. The exponential phase cells in saline were considered fresh cells. The non-culturable cells of *V. vulnificus* were generated by inoculating the fresh cells into artificial sea water and keeping at 2-8℃ for 10-12 days. The non-culturable cells of *E. coli* were generated by inoculating the fresh cells into 7% NaCl at 20-25℃ for 8-10 days.

For scanning electron microscopic (SEM) examination, both fresh and NC cells of *V. vulnificus* and *E. coli* were fixed in 2% glutaraldehyde in 0.1 M buffer. These suspensions were passed through 0.2 μm retention rated polycarbonate membranes. The membranes were rinsed in 0.1 M cacodylate buffer and then dehydrated in a graded ethanol series to 100 % ethyl alcohol (EtOH). They were then placed into solutions of EtOH and hexamethyldisilazane (HMDS). They were then rinsed three times in 100% HMDS. The membranes were then mounted onto aluminum stubs and sputter coated with palladium. The membranes were examined in a field emission scanning electron microscope (JEOL JSM-6300F). Fixed transmission electron microscopic (TEM) samples were osmicated followed by dehydration and embedment in Spurr's resin. Thin sections were stained with uranyl acetate and lead citrate and were imaged on the transmission electron microscope (JEOL JEM-1400).

In a stress condition, cell size generally becomes smaller than in the culturable state. Rod or spiral rod shaped cells of some bacteria can transform to coccoids [1-2]. This alteration in cell morphology was observed in *V. vulnificus* and *E. coli* in our laboratory (Figures 1-6). The fresh *V. vulnificus* is seen in Figures 1 and 2 by SEM and TEM, respectively. In Figures 3 and 4 NC *V. vulnificus* is seen by SEM and TEM showing a “coccoid” morphology with an intact cytoplasmic membrane as seen by TEM. The morphology of the fresh and NC *E. coli* can be seen in the TEM images in Figure 5 and 6, respectively. Since no longitudinal views are seen of the NC *E. coli* (as in the fresh) it is assumed that the NC *E. coli* also has a “coccoid” morphology. An intact cytoplasmic membrane is also shown for NC *E. coli* as in the NC *V. vulnificus*.

References

[1] S. Chaiyanan, et al., Environ Microbiol 9 (2007) 393.
[2] N. Azevedo, et al., Appl Environ Microbiol 73 (2007) 3423.

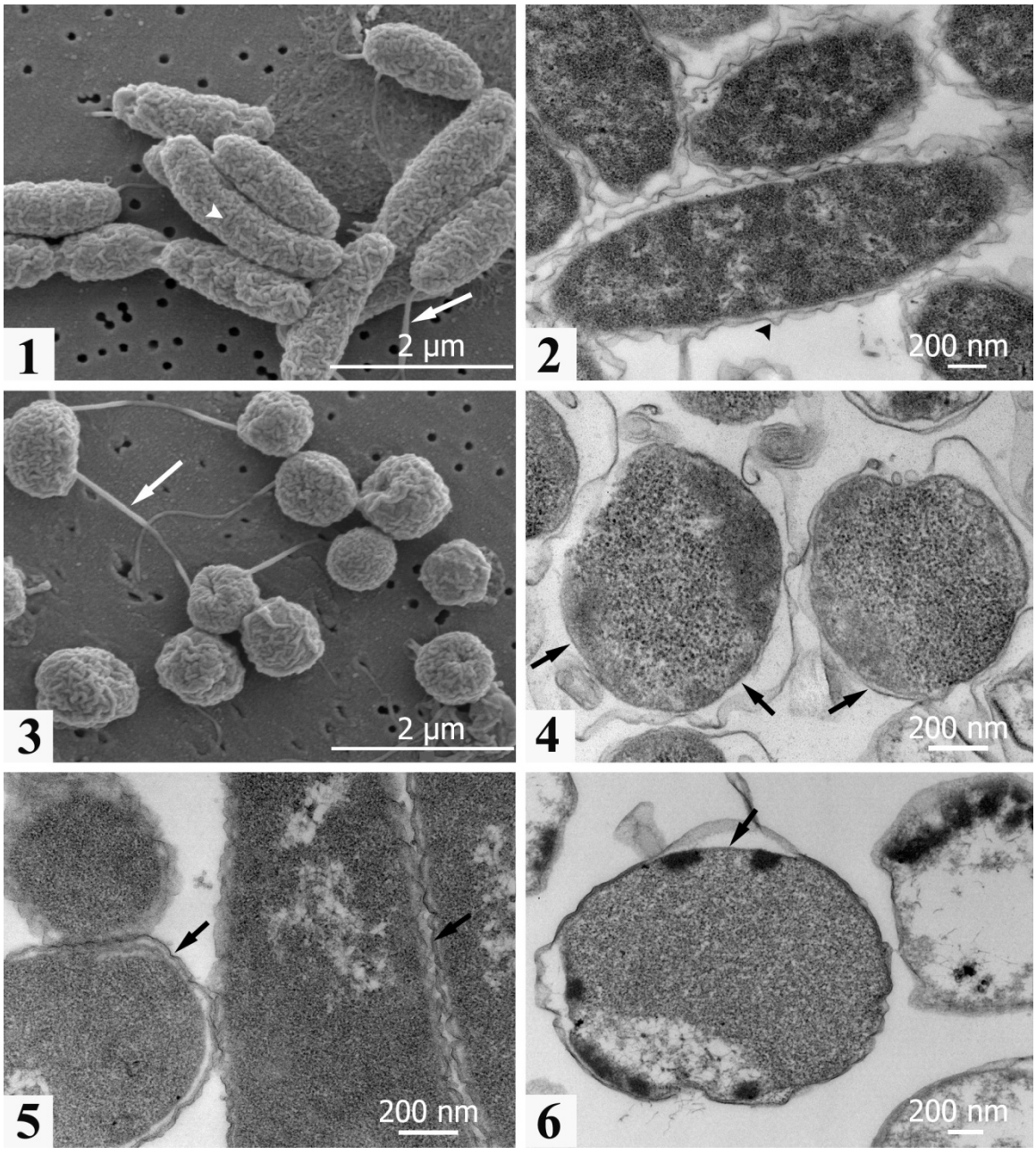

Figure 1. SEM of fresh *V. vulnificus*, capsule (arrowhead) and flagella (arrow).
Figure 2. TEM of fresh *V. vulnificus*, capsule (arrowhead).
Figure 3. SEM of NC *V. vulnificus*, flagella (arrow).
Figure 4. TEM of NC *V. vulnificus*, membrane (arrows).
Figure 5. TEM of fresh *E. coli*, cell wall (arrow).
Figure 6. TEM of NC *E. coli*, plasma membrane (arrow).

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