Area: Microbial Structure

Microorganism: *Campylobacter fetus*

Reference: Graham, L. L., and Feero, S. E. 2019. The *Campylobacter fetus* S layer provides resistance to photoactivated zinc oxide nanoparticles. Canadian Journal of Microbiology 65: 450-460.

The genus *Campylobacter* includes more than 26 species of Gram-negative spiral shaped bacteria that are often pathogenic to humans or other animals. *C. fetus* includes several subspecies that can cause venereal disease in cattle, sheep, or goats, leading to premature labor and spontaneous abortion. While antibiotics can be used to treat these infections, an alternative approach involves the use of zinc oxide (ZnO) nanoparticles which can penetrate the cell envelope and lead to the formation of reactive oxygen species that are highly toxic. Graham and Feero (Can. J. Microbiol. 65: 450-460, 2019) investigated the role of the S layer of the cell envelope *C. fetus* in protecting the bacteria from ZnO nanoparticles. The following picture from their paper shown an electron micrograph of some of these particles.



The following figure is a diagram of the envelope of a Gram-negative bacterium.

Graphical user interface

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1. What is the best definition of a bacterial S layer?

1. It is an organized bilayer of phospholipids and proteins that surrounds the

cytoplasm.

1. It is a multilayered network of peptidoglycan molecules that lies outside of the plasma membrane.
2. It is a second layer of phospholipids and proteins and surrounds the cell wall.
3. It is an ordered array of identcal protein molecules that lies outside of the outer membrane.

To study the effects of ZnO nanoparticles on *C. fetus*, Graham and Feero used several strains of that different in their expression of the S layer proteins. Cff 13783 and Cff 11686 had high levels of expression and Cff 13783K and Cff 11686K had low levels of expression due to insertion of transposable element carrying a gene for kanamycin resistance. The bacteria were routinely cultured on Columbia agar supplemented with 5% sheep blood without or with 50 µg/ml kanamycin. The next figure shows the number of viable bacteria present in each culture expressed as colony forming units/ml (CFU/ml) following exposure to different concentrations of ZnO nanoparticles for up to 6 hours.

Diagram, schematic

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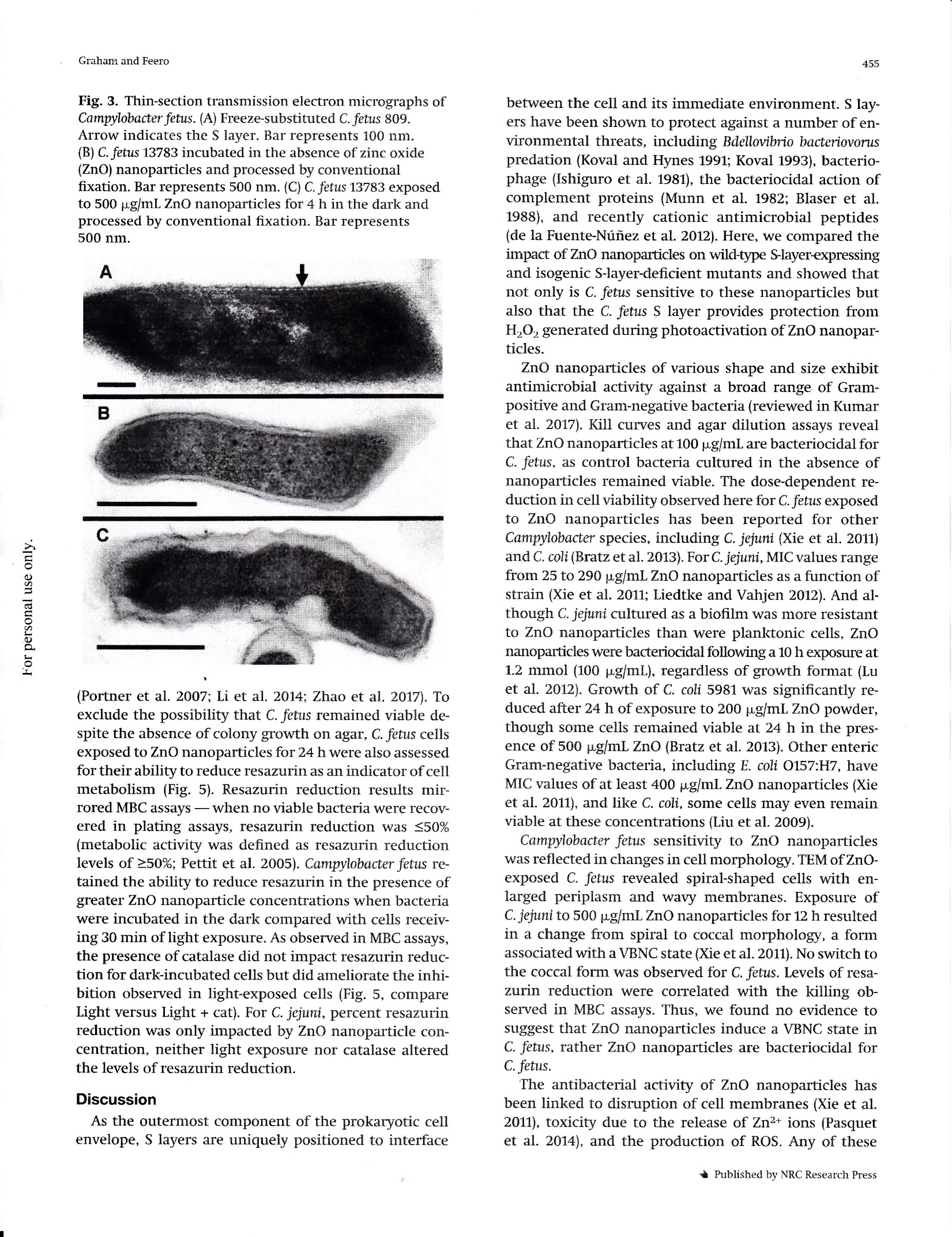
Diagram, schematic

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2. What is the best conclusion to be drawn from these data?

1. There was no decrease in viability in the untreated samples or in any of the treated samples.
2. There was no decrease in viability in the untreated samples but viability was reduced in a concentration dependent-fashion in all of the treated samples.
3. There was no decrease in viability in the untreated samples but viability was reduced in a concentration-dependent fashion more in the strains with an S layer than in the ones without an S layer.
4. There was no decrease in viability in the untreated samples but viability was reduced in a concentration-dependent fashion more in the strains without an S layer than in the ones with an S layer.

To determine if the ZnO nanoparticles might affect the cell envelope of the bacteria, the authors examined thin sections of the bacteria by electron microscopy. The results are shown in the following figure. Panel A shows an image of freeze substituted *C. fetus*, Panelt B shows an image of *C. fetus* not treated with ZnO nanoparticles but processed by conventional EM techniques; and Panel C shows an image of *C. fetus* treated with 500 µg/ml ZnO nanoparticles for four houws and processed by conventional EM techniques.



3. What can you see in these images?

1. The ZnO treated cells have the same general appearance as the untreated cells.
2. The bacteria treated with ZnO nanoparticles have extensive gaps in the cell envelope between the cell membrane and the outer membrane.
3. The bacteria treated with ZnO nanoparticles have no S layer.
4. The bacteria treated with ZnO nanoparticles have no cell envelope and have undergone extensive lysis.

In previous studies, it was found that bacteria treated with ZnO nanoparticles and then exposed to visible light died at a greater rate than those kept in the dark. To determine if this were true for C. fetus, they inoculated medium containing different concentrations of ZnO nanoparticles in the wells of microtiter plates with the same strains used before. One plate was incubated in the dark and the other exposed to light for 30 min. They then determined the number of viable cells after 24 hours and expressed the results again as CFU/ml. In some cases, the enzyme catalase was added at a concentration of 4 µg/ml to remove reactive oxygen species like hydrogen peroxide. The results are shown in the next figure.

Diagram

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Diagram

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4. Look first at the solid line for the cultures that did not contain catalase but that were treated in the dark or the light. What can you conclude from these data?

1. All of the strains showed the same sensitivities to different concentrations of ZnO nanoparticles.
2. The strains that have more S layer proteins (Cff 13783 and Cff 11686) are more senstive to the ZnO nanoparticles than the strains that have less S layer proteins (Cff 13783K and Cff 11686K).
3. The strains that have more S layer proteins (Cff 13783 and Cff 11686) are less senstive to the ZnO nanoparticles than the strains that have less S layer proteins (Cff 13783K and Cff 11686K).

d. For any particular strain like 11783, the expression of the S layer proteins has no effect.

5. Now look at the dashed lines for the cultures that contaned catalase. What can you learn from these data?

a. Catalase has no effect on the senstivity of the bacteria ZnO nanoparticles and light.

b. Catalase makes the bacteria more sensitive to ZnO nanoparticles and light.

c. Catalase makes the bacteria less sensitive to ZnO nanoparticles and light.

d. Catalase only affects the bacteria that are kanamycin resistant.

To determine if these results were specific to *C. fetus*, the authors did similar experiments with another species of *Campylobacter* called *C. jejuni*. The results are shown in the next figure.

Diagram

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6. What can you conclude from this experiment?

a. *C. jejuni* shows the same sensitivities to ZnO nanoparticles and light as *C. fetus*.

b. *C. jejuni* is sensitive to ZnO nanoparticles but only in the light.

c. *C. jejuni* has the same sensitivities to ZnO nanoparticles in both the dark and the

light.

d. *C. jejuni* is not sensitive to ZnO nanoparticles.

7. Based on these experiments, what would be the most interesting experiment to do next?

a. Repeat these experiments with the same concentratrations of ZnO nanoparticles.

b. Repeat these experiments with higher concentration of ZnO nanoparticles.

c. Compare the S layer of *C. jejuni* to that of *C. fetus*.

d. Compare the S layer of *C. fetus* to that of *E. coli*.

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