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Investigating the Role of Membrane Vesicles in Zn-Microbe Interactions

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Membrane vesicles (MV) are extracellular formations derived from the outer-membrane of Gram-negative bacteria, ranging from 50-200 nm in size. Their biological roles include transport of virulence factors, protein and DNA exchange, cell-cell communication, and biofilm formation. In addition, MVs are considered a key vehicle in host-pathogen interactions. Also, because of their high surface area to volume, in the presence of high concentrations of metal, MVs may also play an integral role in modulating metal ion binding to the cell surface by increasing the number of potential metal binding sites. To address additional roles of MVs, we used the Gram-negative bacterium *Burkholderia vietnamiensis* $PR1_{301}$ (PR1) which in humans is involved in co-infecting cystic fibrosis patients. From an environmental perspective, PR1 has been used in bioremediation applications and has previously been studied by our laboratory for its resistance to divalent metals including Zn^{2+}. We have shown that PR1 possesses an uncharacterized pH-dependent mechanism of Zn^{2+}-resistance, and is 10-fold more resistant to Zn^{2+} at pH 5 versus pH 7. Interestingly, we have found evidence that MVs may be playing a role in pH-dependent Zn toxicity. When PR1 is grown in the presence of 100 mg/L Zn^{2+} at pH 7, using SEM/EDX, it was shown that Zn is localized to MVs but is not associated with cells. Additionally, at pH 5 versus 7 in the absence of Zn^{2+}, PR1 produces approximately half as much MVs (2.1 versus 4.1 mg MV protein/L, respectively). A MV purification scheme was then developed using centrifugation, filtration, and ammonium sulfate precipitation followed by density gradient centrifugation. Ongoing studies will characterize purified MVs produced at pH 5 and 7 using shotgun proteomics. These data will provide valuable insight into how the protein composition of MVs at different pH could be involved in Zn^{2+}-microbe interactions as well as provide the first study of the influence of pH on MV protein composition, which may be relevant to studying the host-pathogen relationship.

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