Quantification of Outer Membrane Vesicle Production in *Janthinobacterium* with Arsenic as an Environmental Stressor

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Abstract

Gram negative bacteria have an outer membrane that produces sac-like structures known as outer membrane vesicles (OMVs). OMVs interact with the environment via their cargo, which are typically active proteins. The mechanisms behind this vesiculation are still relatively unknown, yet studies have shown that environmental stressors can boost OMV biogenesis. *Janthinobacterium* isolate #19 is a genus of Gram-negative bacteria isolated from periphyton samples collected from Lake Killarney in Federal Way, WA which has higher arsenic levels than that of other lakes in the area. There is a significant knowledge gap on the *Janthinobacterium* genus, but it does prove to be highly arsenic resistant. To further understand the role of arsenic resistance within *Janthinobacterium* we hypothesized that growing the isolate with the environmental stressor of arsenate (AsV) will result in an increase of OMV production. Supernatant of isolated matrix materials and OMVs were measured using a fluorescent probe assay to indicate the presence of lipid membrane. The results showed no significant change in the production of OMVs in *Janthinobacterium* grown in AsV when compared to our control of *Janthinobacterium* grown on LB medium alone. This indicates that AsV is not acting as a stressor to induce OMV biogenesis supporting the null hypothesis. Future exploration in this research might consider using a minimal medium to identify if AsV uptake competes with the abundance of phosphate found in complex medium. Additionally, because OMV production was not affected using AsV as a stressor, we are looking at the role that *Janthinobacterium* plays in the biofilm community by quantifying the amount of AsV that is taken up into the cell vs the amount of arsenite (AsIII) being reduced and pumped back into the environment.

Background

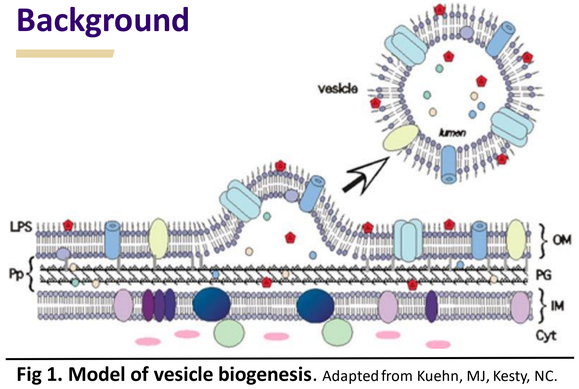


Fig 1. Model of vesicle biogenesis. Adapted from Kuehn, MJ, Kesty, NC. 2005. Bacterial outer membrane vesicles and the host-pathogen interaction.

Methods

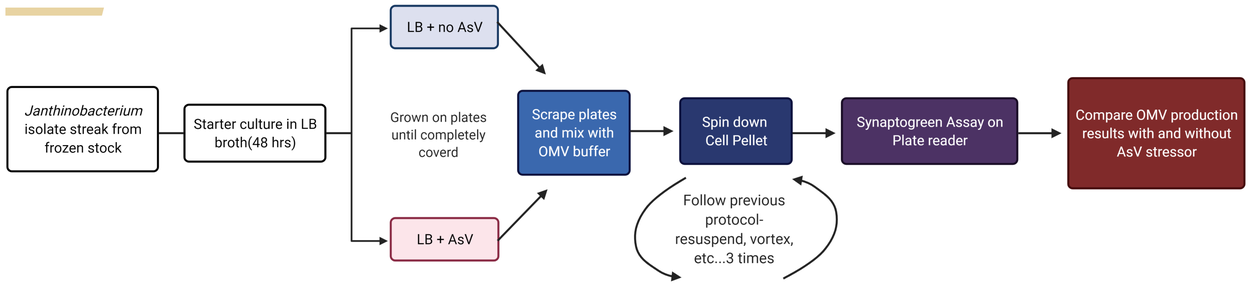


Fig 2. Flowchart of protocol for OMV isolation of *Janthinobacterium sp.* #19 and fluorescent probe assay.

Results

- ***Janthinobacterium sp.* #19 does not show an increase in OMV biogenesis when grown with AsV (50 ug/ml) in LB agar.**
- **Under these specific culture conditions, AsV does not behave as an environmental stressor impacting an increase in membrane vesiculation.**
- ***Janthinobacterium sp*. #19 will grow in high As conditions when grown on complex medium.**

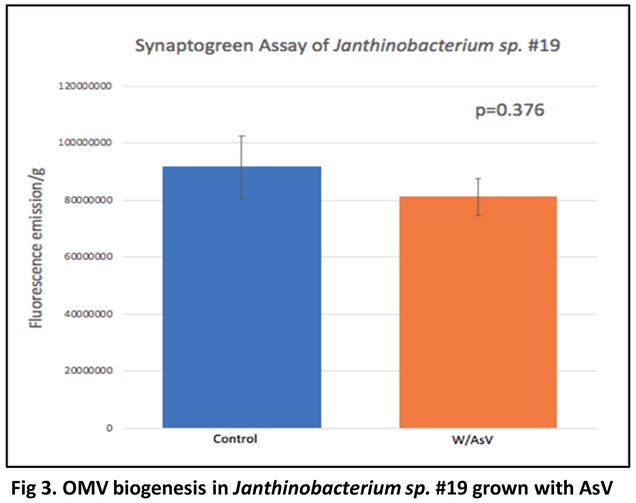


Fig 3. OMV biogenesis in *Janthinobacterium sp.* #19 grown with AsV (50ug/ml) in LB shows slight decrease but is not statistically significant to the control grown on LB agar alone.
Janthinobacterium sp. #19 treated with AsV (50ug/ml) and control with no AsV grown on plates for 48 hrs. Plates were scraped and pellet spun down to isolate OMVs in supernatant, synaptogreen assay measured lipid content assessing change in OMV value. Average of N=9.

Continuing Research

- **Isolate is not affected by high levels of AsV. How much is actually being taken up into cell?**
- **Measurement of AsV uptake by *Janthinobacterium* with increasing [AsV] through digestion assay and ICP-MS.**
- **Preliminary results suggest under lab culture conditions, minimal AsV is taken up by bacteria.**

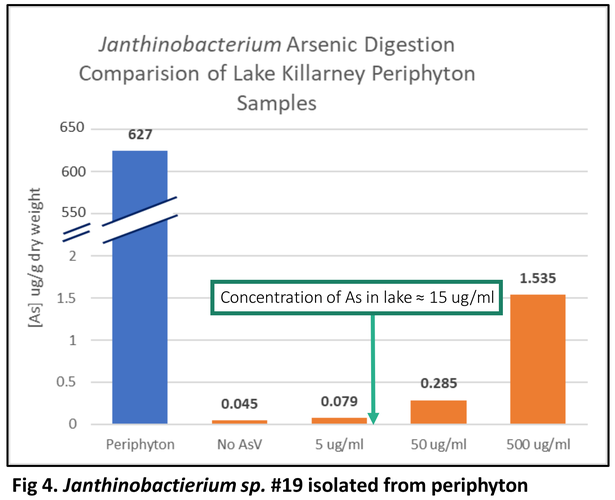
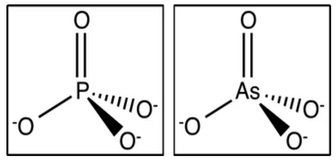


Fig 4. *Janthinobactierium sp.* #19 isolated from periphyton community show little arsenic uptake when grown on increasing [AsV] in LB agar in comparison to the periphyton community.
Janthinobacterium sp. #19 grown on increasing concentrations of AsV in LB agar. Plates were scraped, dried, and digested in 10% nitric acid. Arsenic uptake was measured in parts per billion (ppb) through Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). Dry weight values of arsenic calculated by As(ppb) x dilution volume / digested weight (mg) x dilution ratio. Average of N=3.

Conclusions



Culture conditions do not mimic natural nutrient levels

Fig 5. Similarities of structure between arsenate and phosphate.
Adapted from Parke EC. 2012. What could arsenic bacteria teach us about life?

- **AsV enters cells through phosphate transport systems and may be outcompeting AsV when grown on complex medium.**

Future Direction

- **Alternative defined medias should be explored to allow more AsV to enter cell.**
- **OMV biogenesis and AsV uptake into *Janthinobacterium* may increase with limited phosphate availability.**
- **Culture duration should be increased on both complex and defined media to mimic natural environment.**

References

Kuehn, MJ, Kesty, NC. 2005. Bacterial outer membrane vesicles and the host-pathogen interaction. Genes & development. 19(22):2645–2655.

Parke EC. 2012. What could arsenic bacteria teach us about life? Biology & philosophy. 28(2):205–218.

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