Characterization of OMVs from Brucella abortus 2308

Y. Ortiz and F. J. Sangari .

Department of Molecular Biology, University of Cantabria, IBBTEC, Santander (Cantabria)

Introduction

Outer membrane vesicles (OMVs) were recently described as a new type secretion system. These OMVs are small spheroid particles with size approximately between 20-250 nm of diameter (Perez-Cruz *et al.,* 2013). OMVs contain many different components that are released to the environment to carry out different functions (Deatherage and Cookson, 2012; Dorward and Garon, 1990). Although there has been a historical controversy about their content, it has recently been observed that some bacteria can secrete two different types of OMVs, one formed only by outer membrane and periplasmic components, and a second type that includes also inner membrane and cytoplasmic components (Perez- Cruz *et al.,* 2013; Perez- Cruz *et al.,* 2015). This opens the field to the presence in these vesicles of others cytoplasmic components such as sRNAs. Among the multiple functions of these sRNAs, one is the interference with host functions, as it has been described for the first time for an *Escherichia coli* sRNA acting on *Caenorhabditis elegans* (Knip *et al.,* 2014).

Hypothesis

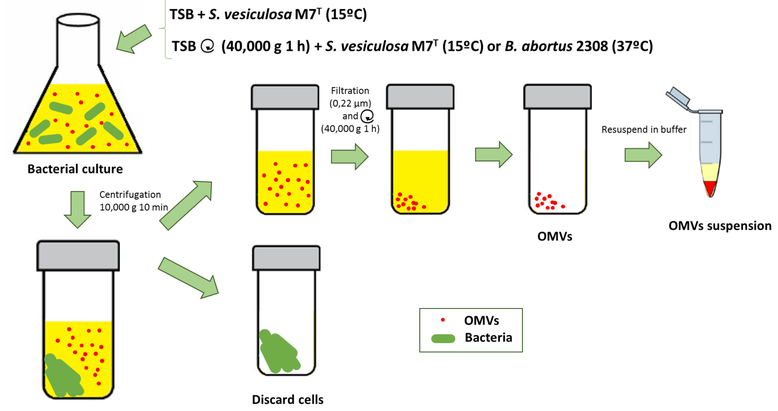
Brucella probably contain both types of vesicles. If so, they could contain sRNAs with the potential of interfering with the host.

Objective

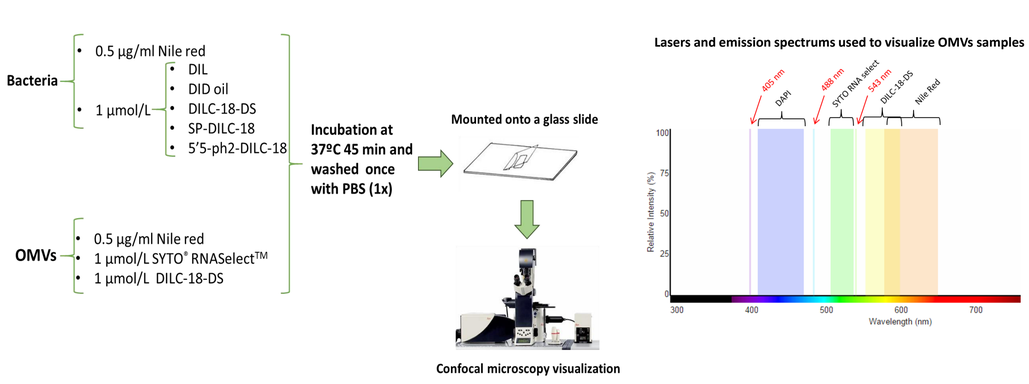
Isolate and visualize *Brucella abortus* 2308 OMVs and their content by different techniques such as confocal and electron microscopy, using *Shewanella vesiculosa* M7^T to standardize all protocols.

Materials and methods

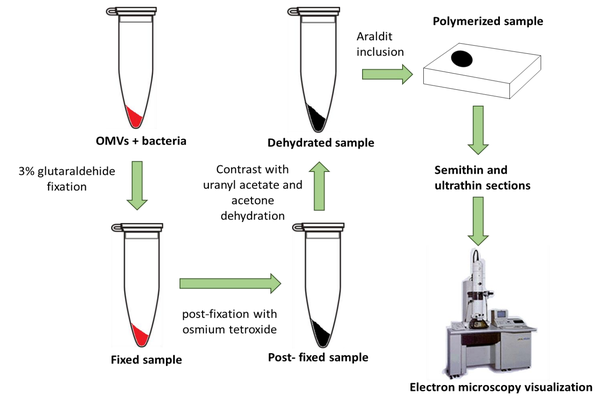
OMVs isolation



Confocal microscopy analysis



Electron microscopy analysis



Results

S. vesiculosa M7^T with different membrane dyes

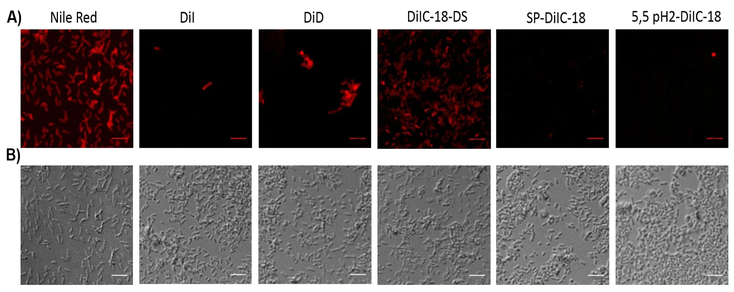


Figure 1. Staining of *S. vesiculosa* M7^T with different membrane dyes. (A) Confocal microscopy analysis from *S. vesiculosa* M7^T stained with different lipid tracer dyes: Nile red, DiI, DiD oil, DiIC-18-DS, Sp-DiIC-18 and 5'5 pH2-DiIC-18. (B) Bright field images of *S. vesiculosa* M7^T stained with different membrane dyes. The scale bar is equivalent to 5 µm in the images.

S. vesiculosa M7^T OMVs with Nile red and Syto® RNAselect™

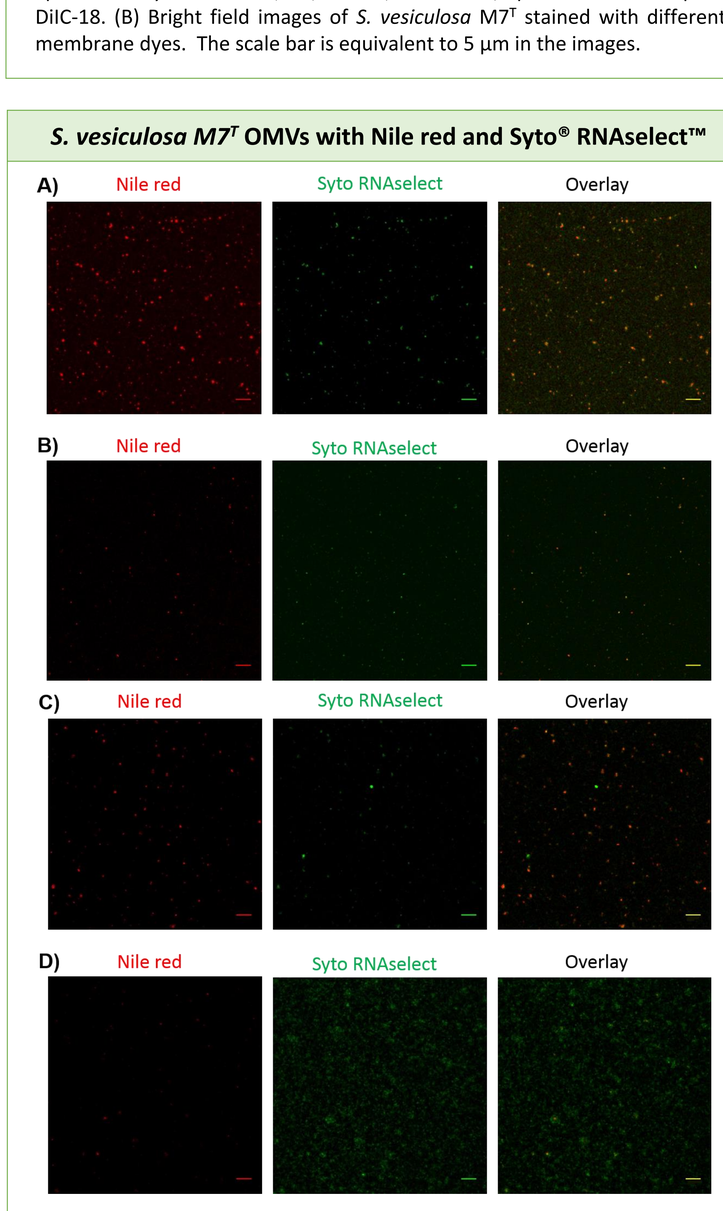


Figure 3. Confocal microscopy analysis of OMVs from S. vesiculosa M7^T stained with membrane dye, Nile red (red) and RNA-specific dye, Syto® RNAselect™ (green). (A) Staining of OMVs obtained in normal medium. (B) Medium control without OMVs. (C) Staining of OMVs obtained in centrifuged medium. (D) Centrifuged medium control without OMVs. The scale bar is equivalent to 5 µm in the images.

S. vesiculosa M7^T OMVs with DiIC-18-DS, Nile red, Syto® RNAselect™

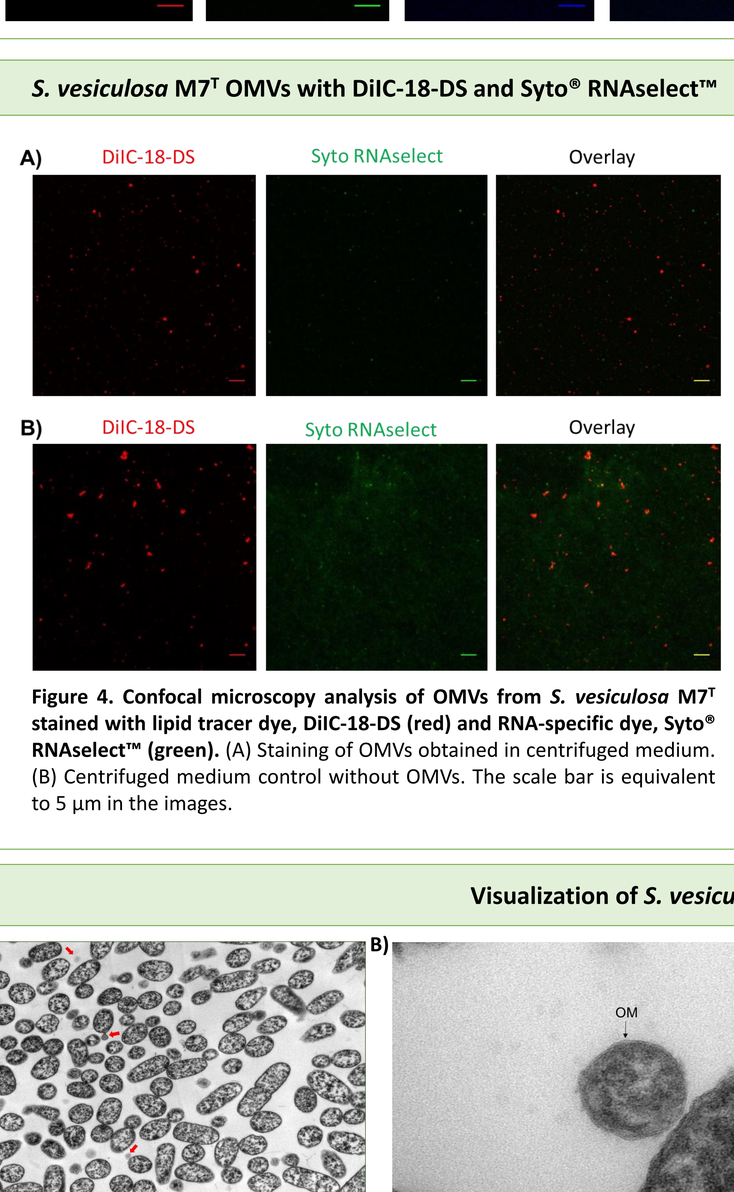


Figure 2. Control of dyes used in confocal microscopy analysis. (A) Control of Nile red emission in others spectrums. OMVs were stained only with Nile red. (B) Control of SYTO® RNAselect™ emission in others spectrums. OMVs were stained only with SYTO® RNAselect™. (C) Control of DiIC-18-DS emission in others spectrums. OMVs were stained only with DiIC-18-DS. The scale bar is equivalent to 5 µm in the images.

S. vesiculosa M7^T OMVs with DiIC-18-DS and Syto® RNAselect™

Figure 4. Confocal microscopy analysis of OMVs from *S. vesiculosa* M7^T stained with lipid tracer dye, DiIC-18-DS (red) and RNA-specific dye, Syto® RNAselect™ (green). (A) Staining of OMVs obtained in centrifuged medium. (B) Centrifuged medium control without OMVs. The scale bar is equivalent to 5 µm in the images.

B. abortus 2308 OMVs with DiIC-18-DS and Syto® RNAselect™

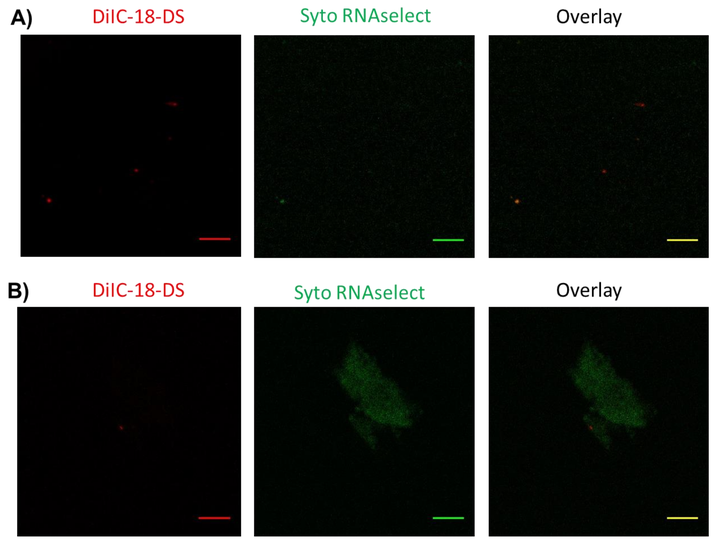


Figure 5. Confocal microscopy analysis of OMVs from B. abortus 2308 stained with lipid tracer dye, DiIC-18-DS (red) and RNA-specific dye, Syto® RNAselect™ (green). (A) Staining of OMVs obtained in centrifuged medium. (B) Centrifuged medium control without OMVs. The scale bar is equivalent to 5 µm in the images.

Visualization of *S. vesiculosa* M7^T OMVs by electron microscopy

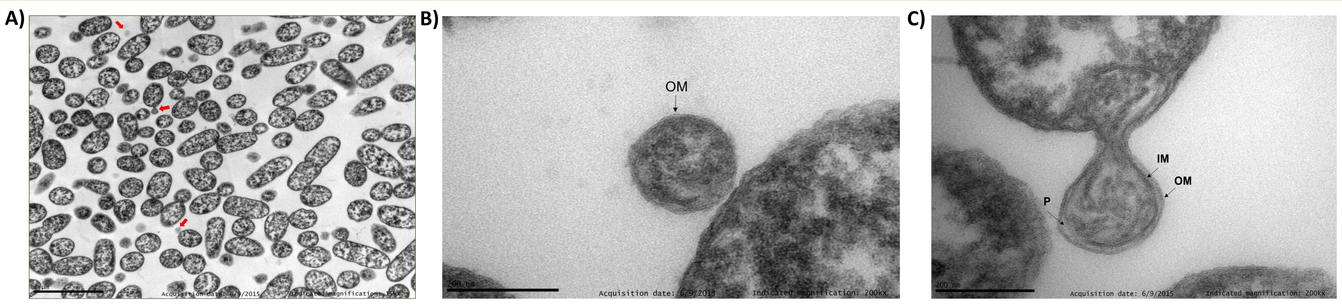


Figure 6. TEM micrographs of ultrathin sections from *S. vesiculosa* M7^T. (A) *S. vesiculosa* M7^T with OMVs. (B) OMV with only one bilayer with the same structure as the outer membrane of the cell. (C) *S. vesiculosa* M7^T secreting a double membrane vesicle. The scale bar is equivalent to 2 µm in A and to 200 nm in B and C. IM. Inner membrane; P: periplasm; OM: outer membrane

Conclusions and further research

- Nile red and DiIC-18-DS are membrane dyes capable of efficiently staining the *S. vesiculosa* M7^T membrane, while DiI, DiD oil, SP-DiIC-18 and 5'5 pH2-DiIC-18-DS are not suitable to stain *S. vesiculosa* M7^T membrane.
- DAPI is not a suitable dye for this experiment because OMVs stained with Nile red, DiIC-18-DS and Syto® RNAselect™ can be detected in its emission spectrum.
- TSB is not a good culture medium to see OMVs by confocal microscopy because it has many fluorescent particles. However, centrifuged TSB is a better alternative because it has less fluorescent particles.
- Confocal microscopy is a simple and rapid method to detect OMVs, as well as the components that they contain, including RNA, but it needs to be improved.
- Confocal microscopy assays suggests that both *S. vesiculosa* M7^T as *B. abortus* 2308 OMVs could contain RNA, but it will be confirmed by other assays, such as electron microscopy or RNA-seq. Moreover, flow cytometry will be used to count the total number of OMVs and the number of them with RNA to obtain the percentage of OMVs with RNA that produces *B. abortus* 2308.
- OMVs with one or two bilayer have been detected in *S. vesiculosa* M7^T by electron microscopy, and we will try to detect and quantify both OMVs types in *B. abortus* 2308.
- Density gradients with Optiprep™ will be used to purify OMVs and thus, try to remove the background in confocal microscopy.
- The work performed with *S. vesiculosa* M7^T has proved to be useful to streamline the work with pathogens like *B. abortus* 2308.

References

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