Foodborne diseases have a high social and economic impact on health care systems and food industries worldwide. Bacteriophages are viruses that specifically infect and kill bacteria. Due to this antimicrobial property, they have been proposed as new tools to improve food safety by controlling pathogenic bacteria in foods [1]. The use of phages as food biopreservatives requires the development of specific formulations that provide stability to the phage particles for their industrial use.

We have previously isolated and characterized the *S. aureus* bacteriophage, phiPLA-RODI, that belongs to the *Myoviridae* family and infects broad range of staphyloccocal species including *S. aureus* from food industry origin [2]. In this work, our aim was to study the viability of this phage (Figure 1) using different encapsulation techniques: emulsions and nanovesicles (liposomes, niosomes and transforesomes), and its stability in nanovesicles during storage along 6 months and under extreme conditions.

### MATERIAL and METHODS

- *S. aureus* IPLA1, was used as the host strain of phase phiPLA-RODI, which was routinely propagated as previously described [2].
- Simple oil-in-water (O/W) emulsions, water-in-oil-in-water (W/O/W) double emulsions and nanovesicles were characterized (Table 1) and the effect of the encapsulation process on the viability of the phage was estimated as the difference between the phage titer before and after encapsulation and expressed as log_{10} reduction in phage titer. Phage titer of phage suspensions after releasing the encapsulated phages was performed by decimal dilution on SM buffer and plated by the double-layer technique using soft TSB medium (0.7% agar) in the upper layer.
- Efficiency of encapsulation (EE) was calculated as the percentage of phages encapsulated inside nanovesicles compared to the total (encapsulated + free or non-encapsulated) phage titer (Table 2). To determine the titer of non-encapsulated phages or free phages, aliquots of nanovesicles were centrifuged and the supernatant was titrated. Then, the supernatant was removed and the pellets were treated with chloroform to release the encapsulated phage and titrated as described above.
- Stability of phage particles inside nanovesicles during storage and under extreme conditions was assessed by comparing the phage titer before and after storage (Figure 3) or treatment (Table 3).

### RESULTS

#### Table 1. Composition, characteristics (size, zeta potential) and stability (measured as log_{10} reduction in phage titer after treatment compared with the initial titer) of vesicles containing phase phiPLA-RODI.

<table>
<thead>
<tr>
<th>Type of encapsulation</th>
<th>Composition</th>
<th>Components</th>
<th>Concentration [mg/mL]</th>
<th>Z-Average [nm]</th>
<th>ζ-Potential (mV)</th>
<th>Viability loss [log units]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple emulsion</td>
<td>Mpglyol 812 PGPR</td>
<td>80.4% (w/w)</td>
<td>4.02% (w/v)</td>
<td>0.98±0.40</td>
<td>-</td>
<td>1.9±0.2</td>
</tr>
<tr>
<td>Double emulsion</td>
<td>Mpglyol 812 PGPR, Tween 20</td>
<td>16% (v/v)</td>
<td>0.8% (w/v)</td>
<td>29±15</td>
<td>-</td>
<td>1.3±0.6</td>
</tr>
<tr>
<td>Niosomes</td>
<td>Pronasome Nio-N™</td>
<td>50 mg/mL, or 50 mg/mL,</td>
<td>0.85±0.12</td>
<td>-</td>
<td>1.0±0.2</td>
<td></td>
</tr>
<tr>
<td>Liposomes</td>
<td>Pronasome Lipo-N™</td>
<td>50 mg/mL, or 50 mg/mL,</td>
<td>1.60±0.17</td>
<td>-</td>
<td>1.2±0.2</td>
<td></td>
</tr>
<tr>
<td>Transforesomes</td>
<td>Phospholipid 90G and Spans 60 (1:1)</td>
<td>50 mg/mL, or 50 mg/mL,</td>
<td>0.55±0.03</td>
<td>-</td>
<td>1.2±0.2</td>
<td></td>
</tr>
</tbody>
</table>

#### Figure 2. Stability (log_{10} titer) of bacteriophage phiPLA-RODI encapsulated in different types of nanovesicles after storage at 4°C for 6 months. (A) Niosomes, (B) liposomes and (C) transforesomes. Phage titer was determined after encapsulation (black bars) and then after storage for 2 months (dark gray bars), 4 months (light gray bars) and a melting (white bars). (D) nan encapsulated on the phase (E) encapsulated phage. Numbers (4, 8, 12 or 16) indicate the concentration of components expressed in mg/mL. Data represent mean ± standard deviation of three biological replicates. Lower case letters indicate differences in stability (*a* vs *b*), *b* vs *c* and *d* vs *e* non comparable

### CONCLUSIONS

- Phage phiPLA-RODI remained stable after different encapsulation processes: simple or double emulsions and nanovesicles.
- Nanovesicles are also suitable candidates for the production of phase-based formulations that will help to maintain phage stability during or under extreme conditions.
- Niosomes are the most interesting nanovesicles for the encapsulation of phiPLA-RODI. Besides offering the greatest protection to the phage particles, they are also less costly than liposomes or transforesomes. Future work will validate the effectiveness of encapsulated phages in a food matrix.

### REFERENCES
