

As we see this spontaneous activity of endolysins against Gram-negative bacteria (that have a protective OM), one important observation should be made. All these studies have tested endolysins against pathogens that were suspended in low ionic strength buffers, which confer a lower stabilization of the bacterial OMs and subsequently enhance the antibacterial activity of endolysins that otherwise could be absent. Therefore, as the tested conditions of endolysins seem to be very restricted, one can expect that the efficacy of these enzymes may not be translated to other *in vitro* environmental conditions and also to *in vivo*.

15.3.3.2.6 Gram-Negative Targeting Endolysins with Enhanced Thermoresistance

The heat treatments commonly used in the food processing industry require that the antibacterials, when needed to be added in the process, are thermoresistant or their activity will be compromised. A few number of endolysins targeting Gram-negative bacteria, especially those targeting problematic bacteria in foodstuff, were found to present different degrees of resistance to high temperatures. The endolysin PVP-SE1gp146, encoded on the *Salmonella* phage PVP-SE1, was the first reported thermoresistant Gram-negative phage endolysin. This endolysin could maintain its activity at temperatures up to 90°C (Walmagh et al. 2012). The endolysin retained its full activity even after being incubated for 1 hour at 80°C and was only completely inactivated after 40 minutes at 100°C. Similarly, another *Salmonella* phage endolysin, Lys68, could only be inactivated when incubated for 30 minutes at 100°C, maintaining 54.7% of its residual activity at 80°C (Oliveira et al. 2014). Circular dichroism analysis demonstrated the ability of Lys68 to refold into its original conformation upon thermal denaturation.

The *Pseudomonas* phage endolysin LysPA26 was able to retain its full activity at 50°C and still has residual activity against OM-permeabilized cells under heat treatment at 100°C (Guo et al. 2017). Endolysins KZ144 and EL188 present high activity up to 50°C, but at 60°C the activity of both endolysins is completely abolished (Briers et al. 2007). As such, the thermoresistance of these two endolysins is very limited.

To some of these endolysins, it was shown that they are able to rapidly refold after unfolding due to high temperatures (Oliveira et al. 2014). This refolding ability, coupled with a stable conformational structure, can be the reason for the high thermoresistance presented by these Gram-negative phage endolysins (Guo et al. 2017).

The high thermoresistance characteristic of these endolysins demonstrates their potential use as antibacterial components in hurdle technology for food preservation (Walmagh et al. 2012), increasing food safety and decreasing the use of chemical preservatives and the intensity of heat treatments, improving food quality and nutritional values. As a result, the use of endolysins in food has the potential to decrease the number of foodborne diseases and