amidases hydrolyze the bonds between MurNAc and the L-alanine, which separate the glycan strand from the stem peptide. Finally, peptidases degrade the links of the peptide stem, which sometimes serve as crosslinks between the cell wall (Oliveira et al. 2012). Some proteins, which have the cysteine–histidine-dependent amidohydrolase/peptidase (CHAP) as a catalytic domain, have been shown to act both as peptidases and amidases (Navarre et al. 1999; Baker et al. 2006; Nelson et al. 2006).

15.3.1 Virion-Associated Lysins (VALs)

In literature, VALs are also known as virion-associated peptidoglycan hydrolases (VAPGHs) (Rodriguez-Rubio et al. 2013b), tail-associated muralytic enzymes (TAMEs) (Paul et al. 2011), and structural lysins or exolysins (Oliveira et al. 2013), which have been discouraged for being misleading terms.

VALs are frequently associated with the phage structural proteins. They are found in many phages mostly associated not only with tail-associated proteins (e.g. *Lactococcus lactis* phage TP901-1) but also with tape measure proteins (e.g. *Staphylococcus aureus* phage φIPLA35), baseplates (e.g. *E. coli* phage T4), and internal capsid proteins (e.g. *E. coli* phage T7) (Lavigne et al. 2006; Nishima et al. 2011). Besides being a small part of a larger structural protein, VALs have been successfully cloned and recombinantly produced in *E. coli* expression systems. Nevertheless, compared to endolysins, VALs have been much less explored in terms of structure, muralytic, and antibacterial activity. First studies started with the characterization of the muratlytic activity of VALs from *L. lactis* phages Tuc2009 and TP901-1 and *Bacillus subtilis* phage φ29, showing their ability to digest the peptidoglycan during the initial phage infection cycle (Kenny et al. 2004; Sudiarta et al. 2010; Mahony et al. 2016). Later studies have validated the muralytic activity of VALs against *S. aureus* and *Pseudomonas* hosts (Caldentey and Bamford 1992; Briers et al. 2008; Lavigne et al. 2006).

15.3.1.1 VAL Structure

While VALs from phages infecting Gram-negative bacteria harbor a single catalytic domain, those from Gram-positive background often encode two enzymatic active domains and with distinct organizations. For instance, *S. aureus* φMR11 VAL was proven to have a bifunctional lytic function by cloning and testing their CHAP and lysozyme individual catalytic domains (Rashel et al. 2008; Briers et al. 2014). A likely explanation for the multi-domain nature of these VALs may be to offer enhanced enzymatic degradation of the thicker peptidoglycan found in Gram-positive host cells.

There are several features that distinguish VALs from endolysins. Structurally, studies have shown that VALs (37–252 kDa) are larger than endolysins (15–40 kDa), with the smallest and biggest being the *Pseudoalteromonas* phage PM2 and *Bacillus* SP-beta prophage VALs, respectively (Kivela et al. 2004;