K64-specific depolymerase successfully enhanced the serum and neutrophil killing *in vitro* and also fully protected K64 *K. pneumoniae*-inoculated mice through depolymerase intraperitoneal injections (18.75 μg per mice) one hour after challenge. Nevertheless, the survival rate significantly decreased to almost 20% when added 8 hours later.

The zoonotic bacteria *P. multocida* is the causative agent of various diseases in intensive animal and can be transmitted to humans usually followed by bites or scratches from domestic animals (Giordano et al. 2015). The capsule A-specific recombinant depolymerase from phage PHB20 that specifically infects *P. multicida* capsular A strains successfully reduced 3.5–4.5 log units of cells in serum (Chen et al. 2018). Moreover, the enzyme successfully rescued more than 60% of challenged mice with a multiple-dose treatment intravenous injected 13 hours postinfection, from an otherwise deadly infection. Also, no pathological changes were observed when compared with the control group. Taken together, depolymerases demonstrated to be efficient in reducing bacterial virulence *in vivo* in animal models by enhancing killing by complement, neutrophils and macrophages.

Other possibilities on the use of depolymerases as antimicrobials have arisen. They have been used to enzymatically degrade and disperse biofilms of *S. aureus* (Gutiérrez et al. 2015) or to be expressed in transgenic pear to reduce *Erwinia amylovora*, the causative agent of fire blight (Malnoy et al. 2005; Flachowsky et al. 2008). Using genetic engineering, depolymerases were incorporated into phage genomes to express them during the phage lytic cycle. This was first demonstrated by engineering phages to express dispersin B, a depolymerase from bacterial origin (Lu and Collins 2007). The synthetic phage was able to reduce biofilms by 4.5 log units, twofold better than the nonenzymatic wild-type phage. This concept was recently translated to an *E. amylovora* phage (Born et al. 2017). While the wild-type phage exhibited poor lytic activity, the engineered phage Y2*::dpoL1-C* had an enhanced performance by combining the capsule–depolymerase activity and the phage lytic activity. This synthetic phage could also significantly reduce the ability of *E. amylovora* to colonize the surface of detached flowers. Finally, the possibility to construct chimeric proteins with enhanced performance is an emerging field of research on phage proteins. One innovative experiment introduced a lytic activity to a depolymerase via fusion with the R-type pyocin (Scholl et al. 2009). This engineered depolymerase became specific and was able to efficiently kill *E. coli* O157:H7 isolates.

15.2.5 Remarks on Depolymerases

The biotechnological application of phage depolymerases is currently unexplored when compared to other phage proteins like endolysins. The first proteins have been characterized from *E. coli*-infecting phages. Since then, many