an exception to the other depolymerases since they not degrade sugars, but instead catalyze the hydrolysis of peptide bonds (polypeptide depolymerases). One example is found in the *Bacillus* phage φNIT1 enzyme, which proved to digest the poly-γ-glutamate-rich capsular polypeptides (Kimura and Itoh 2003). The xylosidase, dextranase, and levanase group of enzymes hydrolyze xylan, dextran, and levan sugars present in bacterial species, respectively. These last three subclasses of enzymes have only been predicted by bioinformatics studies, lacking experimental validation (Pires et al. 2016).

The other main class of depolymerases are the polysaccharide lyases that cleave 1,4 glycosidic bonds via β-elimination mechanism. Three different subgroups of phage depolymerases are found: hyaluronate, alginate, and pectate lyases. Hyaluronate and alginate lyases are a group of enzymes capable of digesting the hyaluronate polymer found in several organisms and the polysaccharide alginate synthetized by bacteria, respectively. For instance, alginate is produced by mucoid *Pseudomonas aeruginosa* strains that are important pathogens in patients with cystic fibrosis.

Lastly, pectate lyases are pectolytic enzymes predicted to degrade the galacturonic acid, one of the major constituents of bacterial polysaccharides. Pectate lyases are one of the most well-known enzymatic group found in phage depolymerases infecting mostly *K. pneumoniae* and *Acinetobacter baumannii* and found to sometimes cleave two capsule types (Majkowska-Skrobek et al. 2016; Hsieh et al. 2017; Oliveira et al. 2017, 2018; Pan et al. 2017; Popova et al. 2017).

15.2.3 Depolymerase Activity Assessment

The *in vitro* assessment of depolymerase enzymatic activity can be accomplished both qualitatively and quantitatively. The latter is more challenging due to the difficulties in separating the K- and O-antigen polymers from the remaining bacterial EPS. Generally, the methods can be divided into biological, biochemical, and physical.

The most common biological method used is the spot-on-lawn (Oliveira et al. 2017). Due to its simplicity and inexpensive implementation, it is usually the first assay used to assess the depolymerase activity. By dropping an enzyme solution into a bacterial host spread on agar plates using the overlay technique, the presence of a halo after a few hours is indicative of depolymerase activity.

The biochemical methods allow an estimation of the enzymatic activity. The turbidimetry assay is the most popular biochemical method and several experimental variants exist. The 3,5-Dinitrosalicylic acid (DNS) assay is used to quantify the number of reducing ends generated upon digestion of the polysaccharide polymer. If combined with glucose as a standard, it is possible to correlate the activity as glucose-reducing end equivalents (Lee et al. 2017). The bicinchoninic acid assay is an analogous method that can also be used to determine the reducing ends in a digested EPS solution (Hernandez-Morales et al.