ABSTRACT

Polysaccharide-degrading enzymes have played an important role in the discovery of biological functional unsaturated oligosaccharides. Although a large number of such enzymes have been reported, the enzymes extracted from actinobacteria that can degrade polysaccharides from seaweed are rather limited. This study was carried out to screen, purify and characterise native enzymes from actinobacteria grown in seaweed media to determine whether actinobacteria can be a good source for producing potentially novel polysaccharide-degrading enzymes. Moreover, the recombinant enzymes were prepared to meet the requirements of commercial polysaccharide-degrading enzymes for industrial processes.

Eighty strains of actinobacteria isolated from decomposing seaweed have been screened for polysaccharide-degrading enzymes. The strains DS40, DS44 and DS79, which were identified as *Streptomyces griseorubens, Streptomyces luridiscabiei,* and *Streptomyces sundarbansensis* respectively, could produce the highest activity of alginate lyase when they were cultured in a seaweed containing medium. The DS40, DS44, and DS79 alginate lyase were purified by combining anion exchange chromatography and size exclusion chromatography with the specific activity of 67.75 U/mg, 108.6 U/mg and 103.4 U/mg, respectively. These enzymes have the molecular weight of approximately 29 kDa. The alginate lyase genes of strains DS40, DS44 and DS79 were composed of 780 bp encoding 259 amino acid residues. There was a 100% similarity in the amino acid sequences of DS40 and DS79 alginate lyase leading to the same type of alginate lyase. DS44 and DS79 alginate

lyase exhibited the activity towards both polyguluronate and polymannuronate, indicated that they are bifunctional alginate lyases, however, they preferentially degraded polyguluronate more so than polymannuronate. The optimal pH of both enzymes was 8.5 and optimal temperatures were 45^{o}C and 55^{o}C for the DS44 and DS79 alginate lyase, respectively. They also were grouped into salt-tolerant alginate lyases and showed optimal salt concentration at 0.6 M. Metal ions Mn^{2+}, Co^{2+}, and Fe^{2+} which increased the alginate degrading activity and by contrast, the enzymes were inhibited in the presence of Zn^{2+} and Cu^{2+}. The highly conserved regions of their amino acid sequences suggested that DS44 and DS79 were alginate lyases of polysaccharide lyase family 7. The ESI- MS analysis showed that the main oligosaccharides break-down products were disaccharides, trisaccharides and tetrasaccharide, which indicated that these enzymes acted as endo type alginate lyases. The amino acid sequence of DS44 showed more than 10% difference to a reported alginate lyase, which demonstrated that DS44 alginate lyase from *Streptomyces luridiscabiei* could be novel and a potential enzyme for efficient productions of alginate oligosacharides with low degrees of polymerization.

The genes encoding DS44 and DS79 alginate lyases were cloned using the plasmid vector pColdI and expressed in *Escherichia coli* BL21 (DE3). The optimal IPTG concentrations for induction at 15^{o}C in the *E. coli* expression system were 0.1 M and 1 M for DS44 and DS79 alginate lyase, respectively. The His-tagged proteins were purified effectively by using a Ni^{2+} Sepharose affinity chromatography and refolded efficiently with a linear gradient of urea (8-0 M). The recombinant DS44 and DS79 alginate lyase exhibited the specific activities of 80.82 U/mg and 78.54 U/mg, respectively. The optimal pH and temperature

of the recombinant DS44 and DS79 enzymes were pH 8.5, 45^{o}C; and pH 7.5, 55^{o}C, respectively. Therefore, cloning and expression of recombinant protein is a powerful technique for producing commercial alginate lyase as a potential candidate for use in various applications in the food, chemical, medical, and biological industries.