Abstract

γ-lactamase is an enzyme clearing five-membered lactam cycles. Polyvinylpyrrolidone (PVP) is one of its potential substrates. Degradation of PVP by γ-lactamase is being studied due to its eventual use in waste-water purifying plants. The aim of the work was to prepare a synthetic gene from the bacterium *Comamonas acidovorans* and to clone it into the expression vector pET22b. PCA method was used for the synthesis of the γ-lactamase gene. 1725 bp long sequence of the γ-lactamase gene was split into two parts (synthons) which were synthesized individually. After the synthesis restriction cleavage and ligation to the vector pUC19 were performed. Competent cells *E. coli*, strain DH5α, were transformed by the obtained construct. After the sequence confirmation both synthons were cleaved by restriction endonucleases and connected by single-step ligation to the plasmid pET22b. Expression bacterial cells E. coli, strain BL21(DE3)RIL, were transformed by the recombinant plasmid containing the connected synthons and expression of the recombinant γ-lactamase was tested. Sequence of the clone producing a protein of the expected length was confirmed by sequencing analysis. The prepared plasmid will be used for the expression of recombinant γ-lactamase. (In English)