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THE CONSTRUCTION OF RECOMBINANT POLYNUCLEOTIDE PHOSPHORYLASE PRODUCING STRAINS

Among the most active and well-studied interferon inducers are double-stranded polyribonucleotides. For polyribonucleotides using polynucleotide phosphorylase (PNPase) that is an enzyme widely distributed among bacteria and catalyzes the polymerization reaction of some natural and modified nucleoside-5'-diphosphates.

PNP is necessary not only to produce interferon inducers, but it can also be used for the synthesis of pharmacologically important polymers containing modified nucleotides.

The aim of this work was to study the possibility of increasing the yield of the reaction catalyzed PNPase polynucleotide synthesis.

We used the bacteria *Enterobacter amnigenus* BIM B-245 as donor gene encoding PNPase, strain *Escherichia coli* BL21 (DE3) as an acceptor of a recombinant vector carrying a gene PNPase.

The PNPase gene was isolated from genomic DNA *Ent. amnigenus* by polymerase chain reaction. The amplification product was separated by electrophoresis on a 1.5% agarose gel, treated with restriction enzymes (*Nde*I and *Xho*I) and ligated into the vector pET24b(+), restriction at the same sites as PNPase gene. *E. coli* BL21 (DE3) cells was transforming the obtaining plasmid. Enzyme purification was performed using affinity chromatography on resin Ni-NTA. Enzymatic polyadenylic acid (poly-A) synthesis was performed using purified PNPase and adenosine-5'-diphosphate (ADP) as substrate. Thus different concentrations of enzyme and ADP were used.

In the result a new genetically engineered strain *E. coli* pET24b-pnp PNPase producer was received.

The results show the availability of recombinant PNPase for polyribonucleotides.