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Molecular cloning of *Pseudomonas aeruginosa* SOD enzyme gene in *Escherichia coli*

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Background and Aims: Superoxide dismutase enzymes (SODs) with various natural sources are metalloproteins characterized by catalyzing the dismutation of superoxide radical to oxygen and hydrogen peroxide. These radical scavengers are responsible to therapeutic effects in treatment of oxidative stress related diseases, and have potent antioxidant properties in cosmetic and food industry against oxidative damages. Recent advancements in biotechnology have focused on producing safer and less expensive enzymes with enhanced potency and specificity. With this concern, we are going to produce recombinant SOD (r-SOD) originated from Pseudomonas aeruginosa, strictly aerobic bacterium with high level of SOD activity.
Methods: P. aeruginosa was cultured on nutrient agar plate. Chromosomal DNA extraction was done by boiling the harvested colonies at 100◦C. Sod specific forward and reverse primers were designed by using sequences submitted in GenBank and computer based alignment softwares. Polymerase chain reaction (PCR) was performed by Pfu DNA polymerase at 62◦C as annealing temperature. Molecular cloning of amplified gene into pET15b vector was done by using T4 DNA ligase and E.coli BL21(DE3), expression host strain. Following confirmation through PCR, restriction enzyme digestion and sequencing analysis, producing the r-SOD is under performing.
Results: Six hundred base pairs sod gene was amplified by PCR. Cloning of amplified gene into pET15b and transformation in E.coli BL21 (DE3) were done successfully. Restriction enzyme mapping and sequencing analysis confirmed recombinant pET15b. Producing the r-SOD protein under IPTG induction is in process.
Conclusion: Superoxide dismutase is among the most potent antioxidants known in the nature. It seems P. aeruginosa aginst SOD with high ability in quenching the reactive oxygen radicals in intense oxidative stress has a potent capacity for genetic engineering manipulation to produce recombinant enzyme for food and therapeutic applications.

Keywords: *Pseudomonas aeruginosa*; Superoxide dismutase; pET15b