Isolation and Cloning of Dsz Operon from Gordonia Sp. in E.coli Expressin Vector

Foroogh Hosseinzadeh*[1]; Mohammad Roayaii Ardakani[2]; Hamid Rajabi Memari[3]

1-Department of Biology,Faculty of Science,Islamic Azad University,Jahrom, Iran
2-Department of Biology, Faculty of Science, Shahid Chamran University, Ahvaz , Iran
3-Department of Agronomy and Plant Breeding, Faculty of Agriculture,Shahid Chamran University, Ahvaz, Iran
hossainzadeh_f@yahoo.com

Background & Objectives: Crude oil contains numerous sulfur compounds in different forms and organic forms (hydrocarbons) are the most important of these kinds of compounds. Combustion of these hydrocarbons result in the release of sulfur dioxid (SO_2) into the environment, that causes air pollution and acid rain.There are two Methods for desulfurization including Hydrodesulfurzation (HDS) which is carried out under high temperature and high pressure and Biodesulfurization (BDS) that is done by microorganisms. BDS is less expensive in comparision with HSD and can be used under normal condition.4S biochemical pathway in microorganisms has the most important role in biodesulfurization.Biodesufurization enzymes are coded by the genes of dszABC operon.The goal of this study was the isolation and cloning of these genes fromGordonia in E.coli expression vector.

Methods: The specific primers for amplification of operon were designed using sequences of operon from Gene Bank.The Genome of Gordonia was extracted by "temperature treatment"Methods and used as template in PCR and PCR product were digested by NdeI and HindIII restriction enzyme and ligated into digested pET-43.1a+.After transformation,recombinant *E .coli* DH5α was spread on selective medium containing ampicillin. Recombinant colonies were checked for presence of the operon by colonyPCR, digestion and sequencing.

Resultes: PCR product band, based on 1Kb ladder was about 3800bp.The digestion of circular vector on agarose gel showed two bands about 1400bp and 5800bp.The product of colony PCR band was in same range of PCR product. A3800bp fragment was isolated from digested recombinant vector.Results of sequencing proved the presence of dsz operon in recombinan.

Conclusion: Isolation and cloning of dsz operon in pET-43.1a expression vector were confirmed by PCR product size, digestion and sequencing. This study provides the possibility of the expression of recombinant dsz operon proteins in *E.coli*. With the optimization of biodesulfurization,it can be used as a bio-alternative methods for Hydrodesulfurization.

Keywords: Cloning; dsz Operon; Gordonia; pET-43.1a