Cloning of Bialaphos Resistant Gene in Expression Vector for Gene Transformation to Dunaliella Salina

Marzieh Siahmard*[1]; Mohammad Hossein Daneshvar[2]; Hamid Rajabi Memary[3]; Mohammad Roayaei[4]; Saeed Kadkhodaei[5]

1-Department of Biothechnology, Ramin University, Ahvaz, Iran

2-Department of Horticulture, Ramin University, Ahvaz, Iran

3-Department of Agriculture ,Shahid Chamran University, Ahvaz, Iran

4-Department of Science, Shahid Chamran University , Ahvaz, Iran

5-University of Putra, Malaysia

marzieh.siahmard@yahoo.com

Background & Objectives: Bialaphos (Phosphinothricin) is an antibiotic as well a non-selective herbicide in agriculture produced by streptomyces hygroscopicus and inactived with phosphinothricin acethyle transferase (PAT) enzyme encoded by bar gene from same bacteria. The bar can be used as a selectable marker in bacteria and plants.Regarding to importance of the bialaphos as a non-selective herbicide in plants, bar gene was cloned in expression vector for transformation and expression of heterologous genes in green microalgae *D.salina* as a host to produce recombinant proteins.

Methods: Bar gene was amplified by PCR, then PCR product was digested with BsrGI and BstEII enzymes and was used in ligation reaction with digested pCAMBIA1305.1 vector. Ligation product was transferred to *E.coli* competent cells, finally recombinant colonies were confirmed by colony PCR, digestion and DNA sequencing.

Results: The PCR product showed the same size of the bar gene(550bp) on agarose gel. Recombinant pCAMBIA1305.1-bar vector was confirmed by both Colony PCR and digestion with AflII and consequently the presence of bar gene in expression vector proved by DNA sequencing.

Conclusion: Regarding importance of the bar gene, the constructed vector can be used in D. salina transformation systems. In this research, the bar genewas cloned in pCAMBIA1305.1 under catalase intron as an expression enhancer sequence to selecte the *D.salina* microalgae in presence of the bialaphos

Keywords: Bialaphos; bar; PCAMBIA1305.1; Streptomyces Hygroscopicus; *Dunaliella salina*